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Good/bad thiols—fine-tuning the balance

Grain orientation effect on extraction of oak flavor compounds

Manipulating light in fruit zone improves grape quality

Functions/benefits of oak barrels

Nitrogen—Friend or foe of red wine quality

Impact of délestge with partial seed removal

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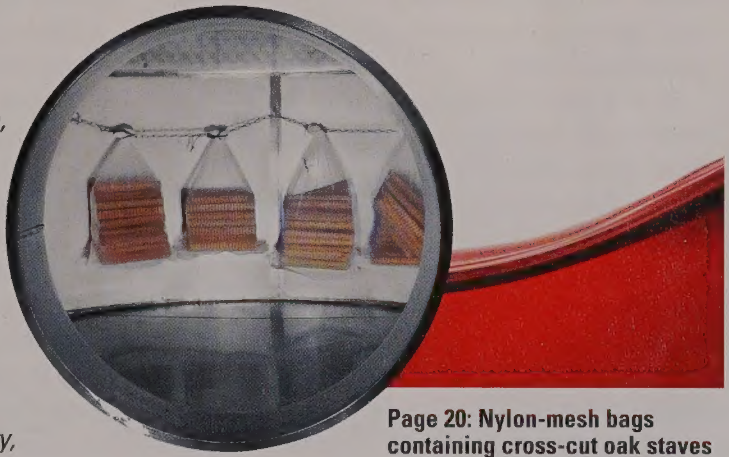

Page 20: Nylon-mesh bags containing cross-cut oak staves hang from a chain around the circumference inside a wine tank.

Page 40: Sunburn on Syrah berries; a common problem with over-exposed grapes and excess nitrogen supply.

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Cover Photo:
Unloading Cabernet Sauvignon grapes into receiving hopper at Ridge Monte Bello winery.
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MANY FACES OF VOLATILE THIOLS IN WINE

Passion fruit with a hint of mineral, some smokiness but kind of stinky

Reductive characters associated with stinky thiols that develop in the wine post-bottling are considered detrimental to perceived wine quality. But preventing their occurrence in a correct way is not straightforward, as we risk to lose more than what we gain. The right dose of oxygen can be the key to fine-tune this complex balance.

BY Maurizio Ugliano, Jean-Baptiste Diéval, Stéphanie Bégrand, Stéphane Vidal,
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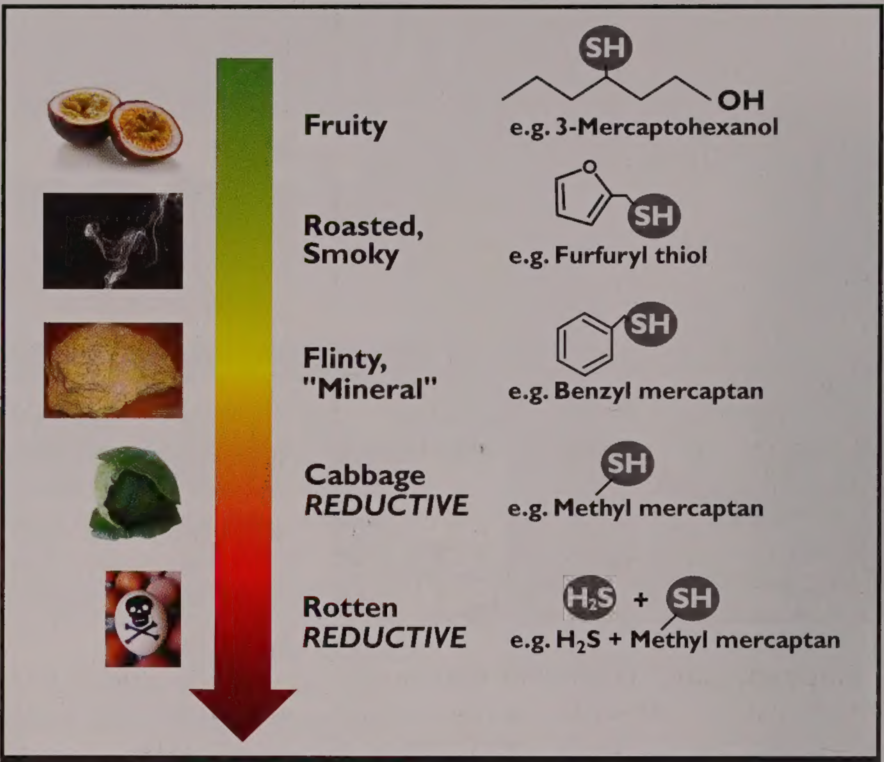


Figure 1: Main volatile thiols in wine and potential sensory contribution from pleasant (green) to unpleasant (red).

Until the early 1990s, volatile sulfur compounds in wine were mainly seen as agents of aroma defects, the only exception being dimethyl sulfide (DMS), which was known to contribute positively to wine aroma when present in low concentrations. As we approached the turn of the last century, however, things changed quite drastically

In the late 1990s researchers at the University of Bordeaux highlighted for the first time the primary role of various grape-derived volatile thiols in the typical aroma of Sauvignon Blanc wines, and this work completely changed our understanding of key aroma compounds in wine.

Today, it appears that nearly every piece of scientific work investigating wine aroma addresses volatile sulfur compounds, which chemists typically refer to as thiols.

At the same time, terms such as "flinty," "mineral," "smoky" and "passion fruit" are becoming a common part of today's wine-tasting jargon. These terms are related to volatile thiols, but certain volatile thiols are also responsible for "reduction," another term long used to designate wines exhibiting unpleasant rotten egg odors.

This article discusses the contribution of volatile thiols to the presence of these aromas in wine, with some thoughts about how winemakers can modulate thiol-related characters by means of controlled oxygen exposure.

Figure 1 shows the different characteristics of the main volatile thiols found in wine. From a chemical perspective, these compounds are all characterized by the presence of the–SH group typical of thiol compounds, which is key to their reactivity in wine, as will be discussed below.

Other volatile sulfur compounds can be implicated in wine aroma, for example dimethyl sulfide (DMS). However, DMS is not a thiol and therefore its reactivity in the wine environment follows different mechanisms than thiols.

Fruity varietal thiols

The compounds 3MH, 3MHA and 4MMP are often referred to as "varietal thiols." This is probably linked to the fact that these thiols were initially identified as key contributors to the aroma of certain grape varieties, in particular Sauvignon Blanc. It was then shown that they contribute to the aroma characteristics of many white and rosé wines, and probably play an important role in some red wines too.

Their presence in wine has been associated with pleasant tropical fruit/passion fruit aromas that seem to be highly sought by today's consumers. Precursors of these extremely potent odorants are in the grapes and can be further formed during must preparation. Yeast activity will then transform these precursors into fragrant compounds.

Therefore, precursor content in grapes and must, yeast strain selection and fermentation management are crucial to the optimization of varietal thiol content in wine.

However, as we will see in more detail below, thiols are, in general, relatively unstable in wine, and therefore post-fermentation and post-bottling factors are equally crucial to wine content of varietal thiols.

Volatile thiols and wine aroma complexity

Today, the definition of specific regional or *terroir* characters seems to be rather topical in the wine community. For example, searching Google for "wine minerality" reveals that seven of the first 10 links speak about minerality in relation to specific wine regions such as Burgundy and the Loire Valley in France, Mosel in Germany or Priorat in Spain, suggesting that minerality might be seen as characteristic of certain *terroirs*.

An exact definition of minerality does not exist yet, and the debate is still open as to whether this is an odor or a mouthfeel attribute. Nevertheless, a recent survey carried out in Burgundy with 2,000 participants made up of wine professionals and wine enthusiasts indicated that "mineral" wines are mainly characterized by struck flint and chalk odors, implying that minerality is essentially an odor attribute.[6]

Benzyl mercaptan, a powerful thiol imparting struck flint aromas (odor threshold 0.3 ng/L), is suspected to play a primary role in wine minerality.[6]

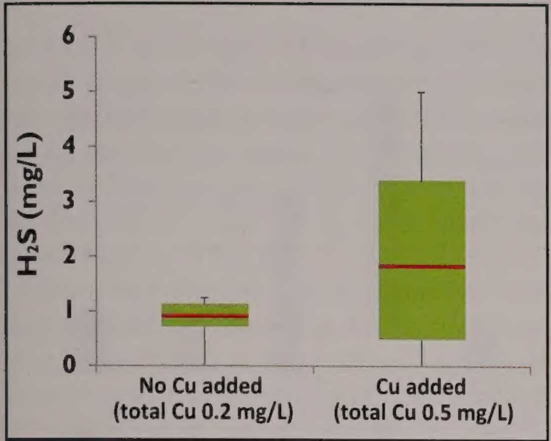


Figure 2: Distribution of H_2S concentration after six months of bottle storage for Sauvignon Blanc wines added or not with copper before bottling. The two groups were significantly different at 95%.

"Empyreumatic" is another term sometimes used to describe complex wine attributes as smoky, roasted meat or toasty bread crust, which can be present in barrel-fermented wines, but also, for wines that have not been in contact with oak, after a certain period of bottle ageing. Such empyreumatic aromas have been associated with volatile thiols such as 2-methyl-3-furanthiol and furfuryl thiol,[7] the latter a key compound in coffee aroma.

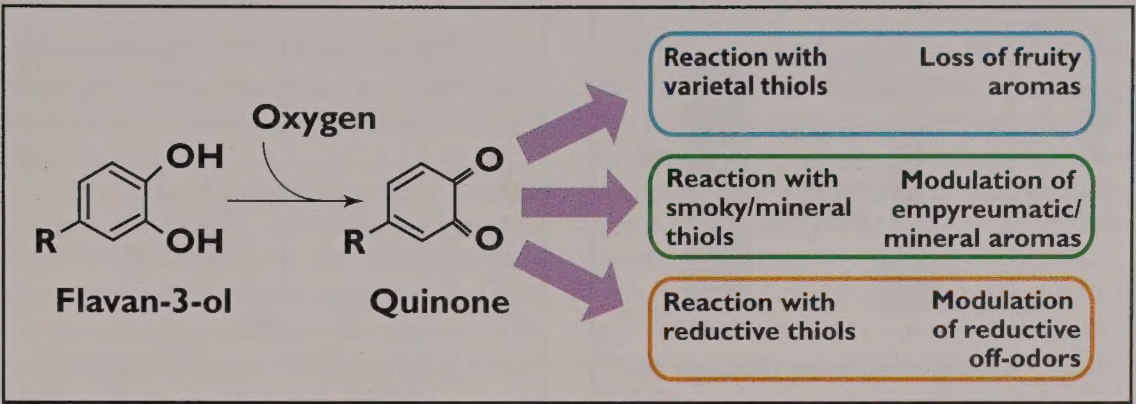

Oxygen
OH
OH
R
O
O
R
Flavan-3-ol
Quinone
Reaction with varietal thiols
Loss of fruity aromas
Reaction with smoky/mineral thiols
Modulation of empyreumatic/ mineral aromas
Reaction with reductive thiols
Modulation of reductive off-odors

Figure 3: Oxygen influence on wine volatile thiols and consequent sensory implications.

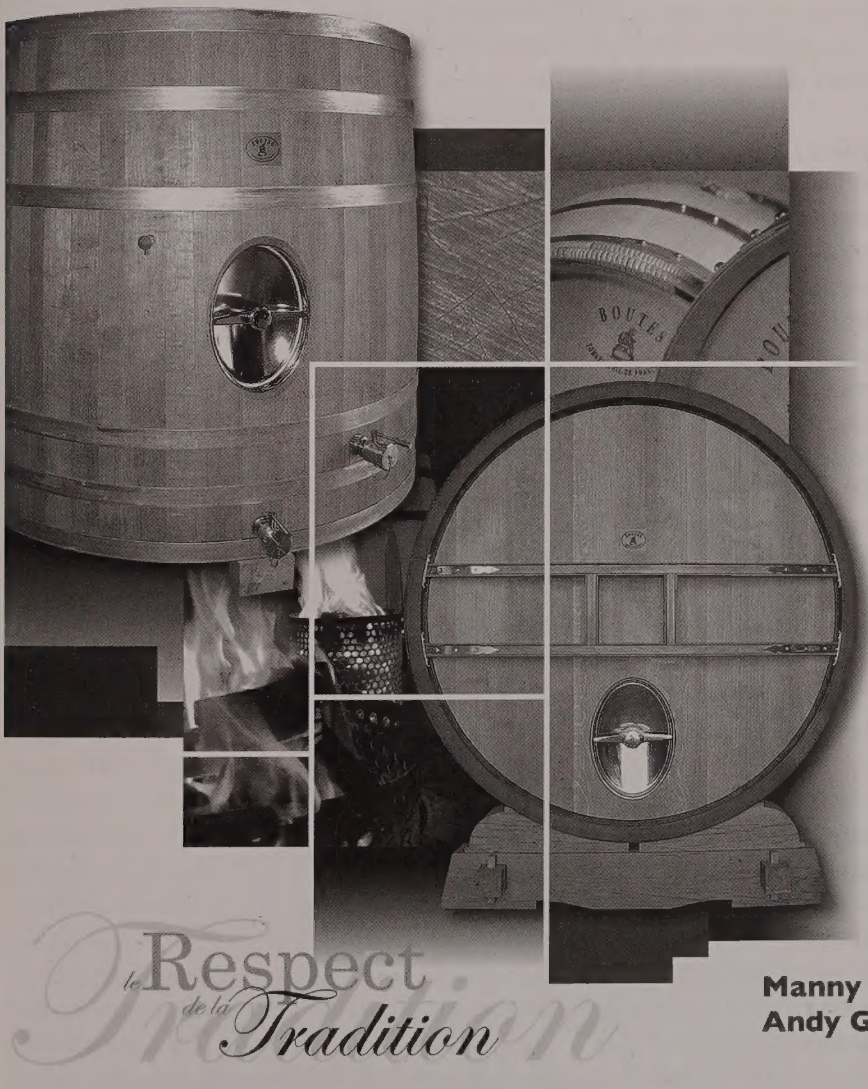
Volatile thiols and reduction

In spite of the potentially favorable outcomes of having certain volatile thiols in wine, there is another side of volatile thiols that is not associated with development of pleasant or complex characters.

"Reduction" is a term often used in wine tasting to designate wines having unpleasant odors of cabbage and rotten egg, which have been associated with the presence of certain thiols such H_2S and methyl mercaptan (MeSH). The origin of these thiols in wine is complex, and their occurrence and concentration depend on multiple factors.

Yeast fermentation is frequently associated with the occurrence of a rotten egg off-odor due to production of H_2S by the yeast. While this reduction can be quite intense, most wines at the end of fermentation exhibit low levels of perceived reduction, and typically low H_2S levels.

Anecdotal evidence indicates that, during further processing and storage of wines in the cellar—for example, during tank or barrel maturation with or without lees—reductive characters might reoccur and require specific intervention to be eliminated. Generally speaking, under the condition commonly adopted in the modern wine industry, most wines are



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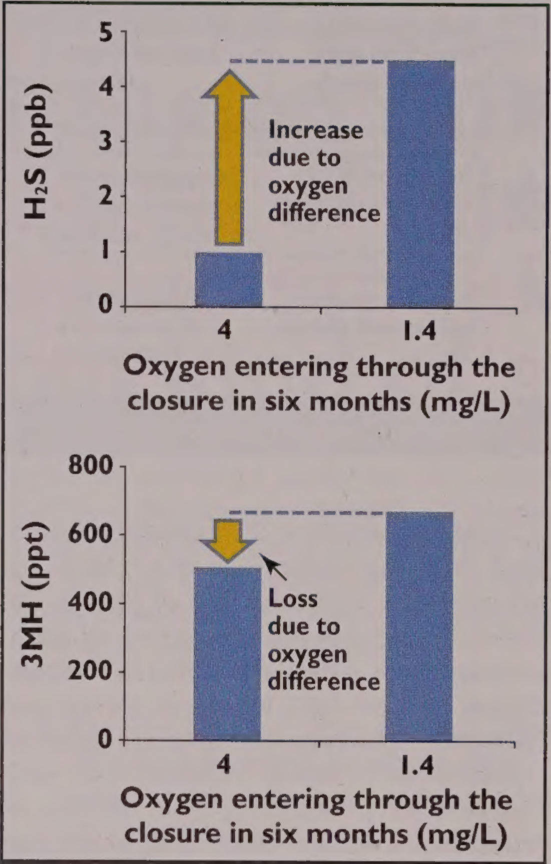


Figure 4: Oxygen influence on two wine volatile thiols during bottle aging of Sauvignon-blanc wine (375 mL bottles).

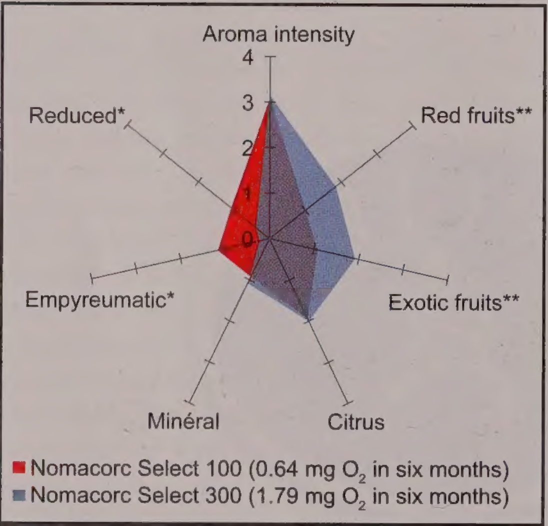


Figure 5: Aroma profiles of a rosé wine after six months of bottle storage with two closures allowing different oxygen ingress. Asterisks denote attributes with significant differences at *95% and **99%.

bottled without any sensorially detectable reductive off-odor.

However, it has long been known that, during storage in bottle, wine can develop reductive aroma characters again, which, from a sensory point of view, appear to be more complex, with descriptors ranging from struck flint to cabbage to rotten egg. This second stage of formation of reductive aromas is of particular concern for winemakers, as it occurs in the finished product that is delivered to consumers.

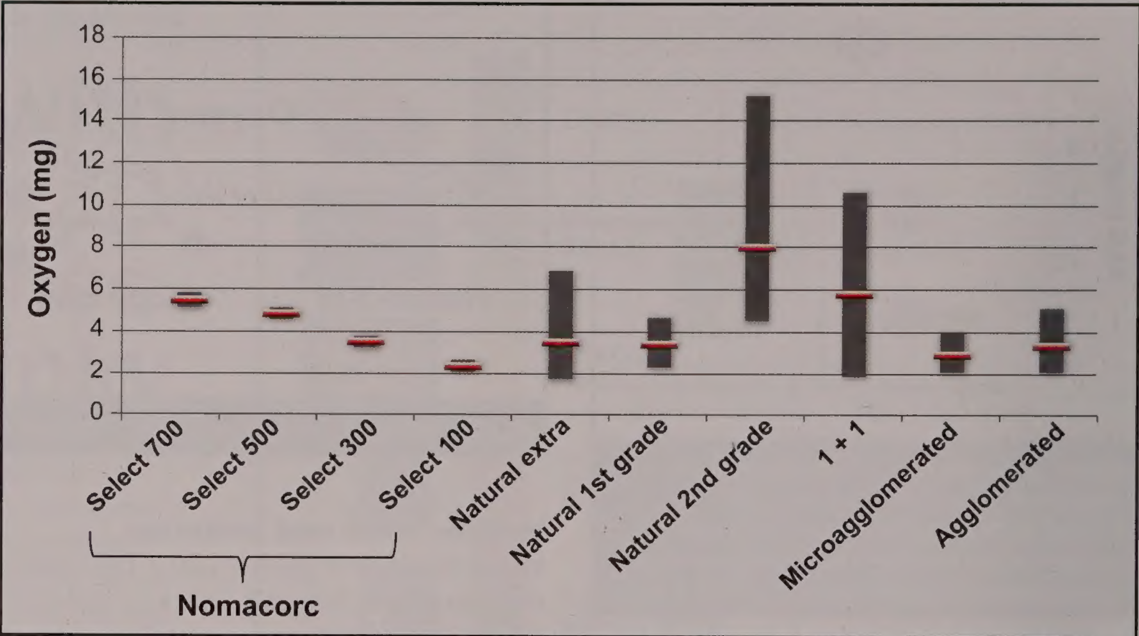


Figure 6: Oxygen ingress value of different closures during two years. For each closure type, measures are taken on 10 closures of a single batch, using a Nomasense oxygen analyzer.

A survey from a major international wine competition indicated that reduction is one of the main wine sensory faults observed in commercial wines.[3]

Origins of volatile thiols and stability during bottle storage

Based on Figure 1, volatile thiols can be implicated in a rather broad array of sensory attributes, ranging from the pleasant passion fruit and grapefruit of certain white wines to the complex smoky/flinty/meaty of bottle-aged wines, but also to the less pleasant reductive odors of MeSH.

Other than having rather different aroma properties, wine volatile thiols also differ for their formation pathways and the winemaking steps that are more crucial to their occurrence. Thiols such as 3MH, 3MHA and 4MMP attain maximum concentrations with fermentation, when the yeast can transform the precursors into the volatile compound.

Although precursors are still present after fermentation, they are stable at wine pH, so that the concentration of these thiols will not further increase during bottle ageing, and indeed they will decline.[8] Means of converting this residual pool of precursors into odor-active compounds, for example by addition of a suitable enzyme, is currently an active area of investigation.

On the other hand, while often not detectable in very young wines, the complex mineral, smoky and roasted coffee aroma characters associated with benzyl mercaptan and furfuryl thiol tend to develop with bottle ageing.[7] Likewise, there is a growing consensus that bottle ageing is also critical for MeSH and H_2S formation.[8,9]

The mechanisms behind this process are not fully understood so far, but it appears that wine contains one or more precursors that can generate reductive thiols during bottle storage.[9,11]

Management of volatile thiols

From a practical perspective, winemakers are typically interested in minimizing the decline of the fruity varietal thiols and, depending on wine style, promoting formation of the smoky/mineral ones. Conversely, they are mostly concerned with excessive accumulation of potentially negative compounds such as H_2S and MeSH.

Unfortunately, as the reactivity of thiols in wine is strongly dependent on the presence of their –SH group, the various thiols often display similar behavior during wine post-fermentation and post-bottling, so that it is not always easy to only favor the "good" ones.

The addition of copper sulfate to remove reductive off-odors or prevent their accumulation during winemaking is probably one of the most commonly used approaches to the management of reductive thiols in wine.

However, as the action of copper on thiols is mostly non-specific, its addition can result in significant losses of 3MH,[8] with negative implications for wine aroma. It can also be assumed that other "good" thiols are removed by copper—not only volatile ones, but also non-volatile ones such as glutathione, a powerful naturally occurring antioxidant.

Moreover, the effect of copper on H_2S is complex, and it was reported that H_2S increases more rapidly during bottle storage, when more copper is present. This can be seen in Figure 2, where wines added with copper at bottling showed much higher H_2S concentrations after six months of bottle storage compared to the same wines that were not treated with copper (the copper addition resulted in an overall increase of copper concentration of only 0.3 mg/L).

The mechanisms responsible for this unexpected behavior are not known, but might be linked to the catalytic action of copper on the breakdown of hitherto unidentified H_2S precursors.[8]

To further complicate this scenario, a radical suppression of all reductive thiols might have unpredictable and, in some cases, negative consequences to wine aroma development after a few months of bottle storage. Indeed, it is possible (although not conclusively demonstrated) that a "bad" thiol such as H_2S might be needed to form potentially "good" thiols such as benzyl mercaptan or furfuryl thiol, meaning that having the bad guys around for a while might eventually have some good outcomes.

Controlled oxygen exposure in the bottle has been shown to be one very effective tool to influence different thiols during bottle storage.[8,9,10,11]

Thiols are very reactive toward the quinones formed upon oxidation of certain wine phenolics, resulting in thiol depletion when wine is exposed to oxygen (Figure 3).

In the case of "varietal thiols," which are formed during fermentation and not replenished from precursors during bottle storage, this means an actual net loss of the aroma compounds, potentially with consequences for the wine's fruity aroma.

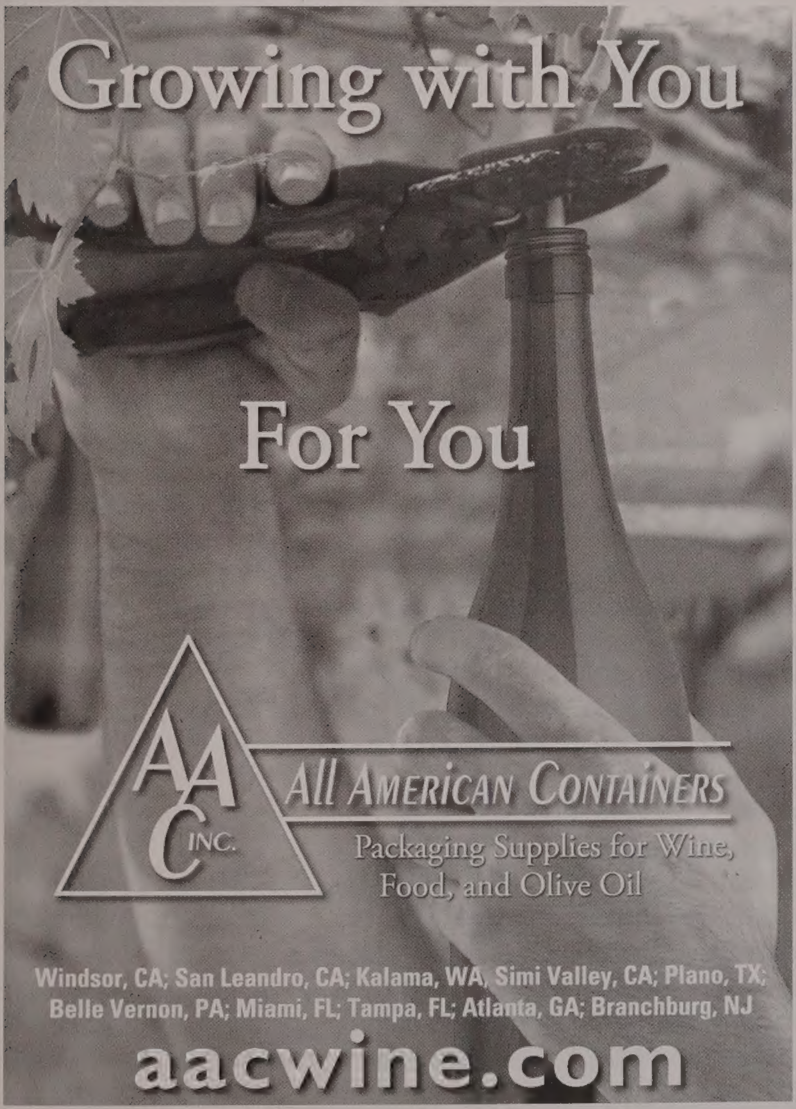
However, in wines with a tendency to form reductive thiols due to their intrinsic characteristics (presence of suitable precursors deriving from yeast metabolism), oxygen exposure will act to soften this potentially negative character.

Luckily, recent data have shown that H_2S seems to be more responsive than 3MH to increased oxygen exposure in the bottle through closures (Figure 4), suggesting that, within certain limits and depending on wine type, oxygen management in the bottle can be used to prevent excessive H_2S increase with relatively minor loss of 3MH. This can be explained by the fact that H_2S has greater reactivity toward quinones than 3MH. Likewise, furfuryl thiol also has higher reactivity than 3MH toward quinone.[4]

With regard to what we have called smoky/mineral thiols, little is known of the factors influencing compounds such as benzyl mercaptan and furfuryl thiol during bottle ageing and on the complex smoky/empyreumatic characters that seem to be linked to these compounds.

In a study involving an independent panel of wine-tasting experts, we recently observed that oxygen permeability of the closure can drastically influence the balance of empyreumatic, reductive and fruity aroma attributes of wine after a period of bottle ageing (Figure 5). In this specific study, the rosé wine showed significantly more intense fruity attributes when exposed to more oxygen, while decreasing oxygen exposure resulted in higher reductive and empyreumatic notes. These observations demonstrate the complex contribution of volatile thiols to wine aroma.

Although a larger degree of oxygen exposure in the bottle is expected to decrease the concentration of fruity varietal thiols, overall the wine expresses more intense fruity attributes, as these are not masked by the presence of excessive reductive and empyreumatic thiols. Conversely, the latter become more dom-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inant when oxygen exposure is lower. Overall, these observations seem to confirm that reductive and empyreumatic thiols are more responsive to oxygen exposure than fruity thiols, consistent with Figure 3.

From a practical perspective, it is important to emphasize that the amount of oxygen needed to shift a wine aroma profile from fruity to more reductive or empyreumatic characters is rather small. This can be seen in Figure 5, where approximately 1 mg of oxygen was already sufficient to obtain these two distinct profiles after only six months in the bottle. In order to modulate the different thiol-related characters according to wine style, it is therefore necessary to precisely control oxygen ingress within a rather narrow range.

Figure 6 shows data relative to oxygen ingress of different types of closures, expressed as the sum of oxygen released from the inner cavities of the closure after bottling compression (a process often referred to as outgassing), plus the oxygen entering through the closure due to its intrinsic permeability (commonly referred to as oxygen transmission rate or OTR).

While OTR is commonly taken as the reference parameter for assessing the potential influence of a closure on a wine's post-bottling development, outgassing should not be neglected, as it can account for a significant portion of the oxygen entering the bottle in the first year (up to 50% or even higher depending on closure type), therefore influencing wine thiol composition even in the short-term.

For example, we have observed that the amount of oxygen derived from desorption alone can account for a 50% loss of 3MH in six months of bottle storage (in 375 mL bottles).

Consistent with other studies, the data in Figure 6 confirms that oxygen ingress is strongly dependent on closure type. However, for certain types of closures it appears not possible to define a specific oxygen ingress value, due to the large variability observed across the single batches analyzed. This is the case for natural cork and 1+1 closures, in agreement with the findings of other studies.[2,5]

Other types of closures are generally more consistent, although variations in the range of 2–3 mg of oxygen over two years were still observed in the case of agglomerated or micro-agglomerated closures.

Conversely, co-extruded synthetic closures (Nomacorc Select series) displayed the most consistent oxygen ingress values, therefore offering improved performances in the management of volatile thiol evolution during wine bottle storage.

Take-Home messages

- Volatile thiols contribute to both pleasant/complex and negative/reductive aroma attributes.
- Removal of reductive thiols by copper is likely to negatively impact fruity thiols. Moreover, copper addition at bottling can enhance H_2S formation during bottle ageing.
- Selection of closure with optimal oxygen ingress can allow removal of excess reduction.
- A co-extruded synthetic closure allows precise and consistent oxygen ingress post-bottling, reducing the risk of bottle-to-bottle variation in wine thiol profile.

Conclusions

Volatile thiols are powerful aroma compounds with a primary role in wine aroma composition. Their sensory contribution is rather diverse, ranging from pleasant passion fruit/exotic fruit (3MH and 3MHA) to complex smoky/mineral (furfuryl thiol and benzyl mercaptan), to unpleasant reductive (H_2S and MeSH) notes.

Bottle ageing is crucial to formation and degradation of different volatile thiols, but achievement of an optimal balance remains challenging. Although often adopted to remove unwanted reductive aromas, copper addition also removes pleasant aromatic thiols and can have unpredictable effects during bottle ageing, including an increase in H_2S accumulation.

Careful selection of closure oxygen ingress can allow winemakers to manage the different aroma nuances associated with varietal thiols, representing a valuable solution for management of wine volatile thiols.

While preservation of volatile thiols generally requires closures with relatively low oxygen ingress, unpleasant reductive thiols seem to be the most responsive to oxygen, increasing the risk of reductive notes with closures allowing too little oxygen.

In addition, a non-negligible variability of oxygen ingress values exists for certain types of closures even within the same production lot. This could lead to significant bottle-to-bottle variation and should therefore be carefully considered for wine styles that are closely dependent on volatile thiols.

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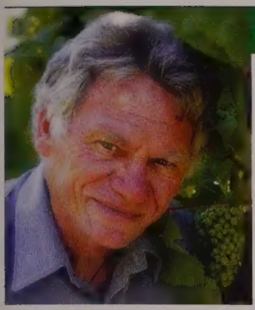


SMART VITICULTURE *by Dr. Richard Smart*

Grapevine trunk diseases: A larger problem than ever posed by phylloxera?

"Trunk Disease, a New Global Vineyard Threat" was the theme of my spring 2012 column. The *PWV* editor inserted a box called "California Input" written by Paul Verdegaal, University of California farm advisor. This comment on my column contains some familiar sentiments made by people around the world explaining poor performance of newly planted vineyards.

The "young vineyard decline" of some new vineyards in California was blamed on issues such as "improper planting, replanted vineyards, pushing vines to produce early, deficit irrigation" and extraordinarily "spur pruning" (hardly a new practice in California!), as perhaps contributing to the problem. These assertions may be true, but I for one am very skeptical and far from being convinced.

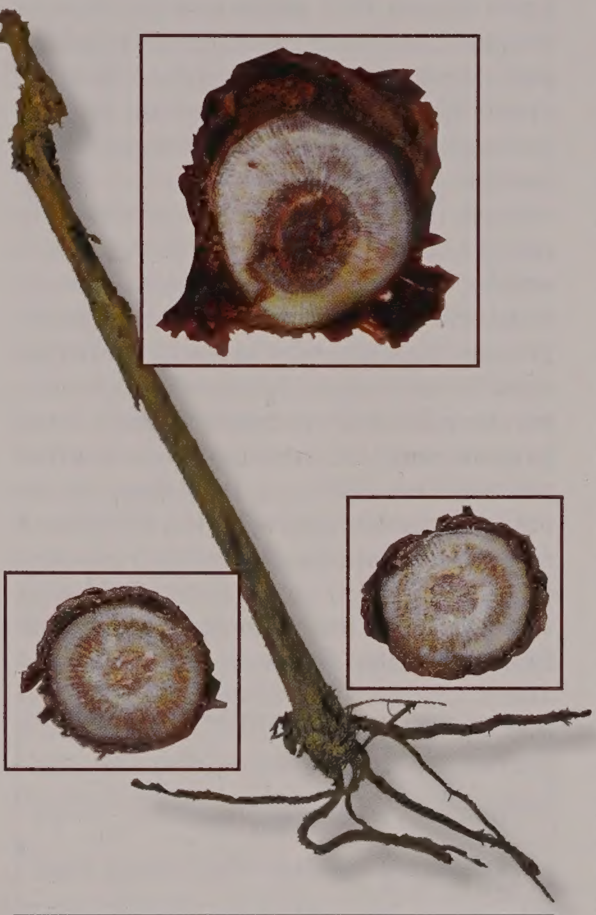
The question remains: Would these stresses be important if the young vines planted were healthy and not affected by trunk disease? Diseased plants usually are more susceptible to stress, trunk disease included.

I understand there remains a reluctance of some California nursery operators (and others worldwide) to admit that some of the vines they are selling can be infected with trunk disease, which can jeopardize the establishment and growth of young vineyards. **Infected vines may not be fit for planting purposes!**

My column followed up a text written by Dr. Doug Gubler and others from the University of California in the January/February 2005 *PWV*, where they discussed grapevine trunk diseases in California. Most of the Gubler *et al.* content was about the then well-recognized *Eutypa* disease, but the end of the report included some new research about "Bot canker," caused by a group of *Botryosphaeriaceae* fungi. A lot has occurred in the eight years since this report was published in *PWV*.

Present situation in California

Gubler's Ph.D. student (by then co-author, José Úrbez-Torres from Spain) and others published in 2006 an extensive survey of California vineyards aiming to identify the species of *Botryosphaeriaceae* associated with canker symptoms in California vineyards.[1] In the past, grapevine canker and consequent dieback symptoms had always been associated with *Eutypa*.



Trunk disease symptoms in a one-year-old grafted vine exported by a California nursery.

The 2006 survey was extensive, including 1,735 samples from 166 vineyards located in 21 counties. *Botryosphaeriaceae* species were found to be surprisingly widespread, being present in 148 of the 166 vineyards sampled, around 90%. In fact they were more abundant than the *Eutypa* dieback causal fungus *Eutypa lata* in the grapevine cankers tested. There were also seven *Botryosphaeriaceae* species discovered. The authors concluded that *Botryosphaeriaceae* may be a more important cause of grapevine dieback than was previously recognized.

Úrbez-Torres and Gubler published a 2009 study of the pathogenicity of the nine *Botryosphaeria* species in California.[2] They showed that all nine species were able to infect both young and old vines, causing cankers, discoloration and streaking of the wood. However, there was a difference in virulence between the species from very virulent to weakly virulent, which indicates that the species of *Botryosphaeria* present in a vineyard may be a very important consideration. The most virulent species are *Botryosphaeria rhodina* (aka *Lasiodiplodia theobromae) and Botryosphaeria lutea* (aka *Neofusicoccum luteum*).

A 2011 study by Úrbez-Torres looked at susceptibility of grapevine pruning wounds to two of the more virulent strains of *Botryosphaeria*.[3] Experiments were done in the field using Chardonnay and Cabernet Sauvignon in the Napa Valley. Vines were pruned at different times and the pruning wounds inoculated or not, up to 84 days after pruning.

Results showed that pruning wound susceptibility was highest in November and lowest in March. Infection was highest immediately after pruning and declined with the age of the wound, but less so than for *Eutypa*. Based on this research and similar studies in California about Eutypa dieback, ideally growers should avoid wet weather when pruning to reduce risk of infection, but this is not always possible.

Other studies have confirmed that the trunk disease situation in California may be more serious than was previously understood. For example P.E. Rolshausen and others published a 2010 study of pruning wound susceptibility to grapevine trunk diseases.[4] The *Botryosphaeria* species tested had the highest infection rates of recent pruning wounds around 80%, and greater than *Eutypa* at around 30%.

These results highlight the potential threat of these pathogens if they become established in production vineyards. Very importantly, the authors also encourage the protection of pruning wounds for mother-vine source blocks used for plant material production in nurseries. These have been found worldwide to be the major source of contamination of young plants produced by nurseries.[12]

Investigations continue about the role of protective sprays for pruning wounds. The practice of "double pruning" developed at the Gubler laboratory enables growers with large acreage to reduce infection by *Botryosphaeria* and *Eutypa* by a follow-up pruning in the spring with less rainfall.

Elsewhere in the world

More recent studies have shown the presence of significant trunk diseases in Arkansas and Missouri, due to 15 different fungi, by Úrbez-Torres and others.[5] These were all new reports, and highlight that grapevine trunk diseases can affect grapevine health of inter-specific hybrids and *vinifera*.

In a 2009 study by Úrbez-Torres and others,[6] *Botryosphaeriaceae* fungi were the most common fungi isolated from grapevines affected with dieback in Texas. The highlight of this study was to show that *Phomopsis* was demonstrated as a trunk disease pathogen able to cause cankers. All fungal species tested were the first reported in Texas.

In the northeast U.S., studies published in 2013 have shown that two species of *Phomopsis*, and one of closely related *Diaporthe eres* were responsible for wood cankers and pruning wound infestations.[8] *Diaporthe eres* has recently (2012) been shown as a grapevine trunk disease in Croatia.[9]

It is a similar picture in Mexico. Úrbez-Torres and others in 2008 reported two *Botryosphaeria* fungi as the primary cause of grapevine dieback and canker formation, and confirmed their pathogenicity.[7]

An Australia study published in 2010 included a survey of 73 vineyards in two states and samples taken from 2,239 grapevines.[10] The most common species found was *Botryosphaeriaceae*, and species varied with region.

There is a survey by Úrbez-Torres and others in British Columbia,[11] where growers and scientists have only recently (since 2007), become concerned about trunk diseases. They surveyed young and old vineyards in 2011, using 173 vineyards, and identified trunk disease as a previously unknown problem. All vineyards sampled showed trunk disease symptoms, including both young and old vineyards.

The situation regarding production of nursery vines is equally serious. The two Spaniards David Gramaje and Josep Armengol published an extensive 2011 review of trunk diseases in the propagation process.[12] They conclude that fungal trunk diseases are a major cause of young vine decline worldwide, which reduces productivity and longevity. These diseases may be called Black Foot or Petri ("Black Goo") disease, and a number of fungal species are involved. These are species of *Cylindrocarpon, Ilyonectria, Phaeoachremonium, Phaeomoniella, Cadophora* and *Botryosphaeriaceae*.

Many investigations around the world have shown that the major problem is fungal infection of cuttings taken principally from diseased rootstock mother vines. Contamination spreads further during the propagation process, especially in water baths.

Such infections can be avoided by using hot water treatment (HWT), as is employed by a couple of Australian nurseries. Why is it not more widely used? Nurserymen around the world are frightened of the process, but new research has produced safer protocols. There is an urgent need for more international research on HWT, so that the process may be more widely adopted. HWT and replanting of rootstock mother vines and preventing their infection will largely overcome the problem of selling trunk disease-infected vines.

> "I agree that we do not have enough information as how to prevent Bot canker in existing vineyards—what matters most to growers," says **Steve Thomas**, vineyard manager at Kunde Family Estate in Kenwood, Calif. "We rely on the expensive painting of pruning cuts in 550 of our 600 vine acres in Sonoma Valley. We spend about $150 per acre painting cuts with paint plus a fungicide. We feel this is worth the expense to extend the life of the vineyard."

What can we conclude from all this?

- There has been much research activity since 2005, in California and elsewhere, which demonstrates that trunk disease by *Botryosphaeriaceae* is a widespread, typically misdiagnosed and an unrecognized problem before recent studies listed above.
- Some diseases, especially *Botryosphaeria*, can be virulent pathogens. They spread by infecting pruning wounds, which they do more successfully than does *Eutypa*. *Botryosphaeriaceae* is presently very widespread in California, found in around 90% of vineyards tested.[1]
- Worldwide, there is a serious problem with infected grapevines produced by nurseries, which causes early and enduring mortality, uneven establishment and variable vineyards. Further, infectious diseases such as *Botryosphaeria* dieback can infect healthy vines and the economic viability of vineyards can be threatened.

> "In my experience, canker diseases are seldom misdiagnosed or unrecognized," says **Paul Verdegaal**, UC Extension farm advisor. "They are, however, sometimes ignored for one of two reasons: either the impacts of the disease are not fully appreciated or, more commonly, growers are uncertain as to how to best manage infected vineyards."

My take on all of this

- I can find no reference to recent extension activities on trunk diseases. There seems much less research on trunk disease than 10 years ago. I reviewed the

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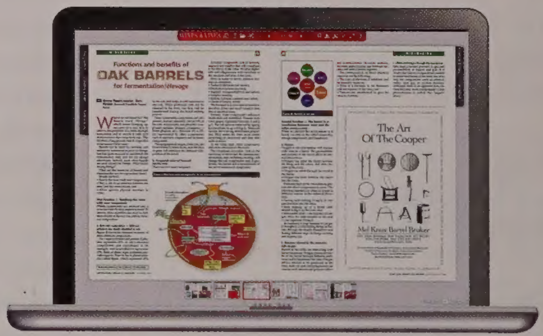
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American Vineyard Foundation-funded projects for 2013-14, 2012, 2011 and 2010, totaling 95. Of these, 42 were related to grapevine pests and diseases including breeding, and of these only two were about grapevine trunk diseases.

- Of the National Grape and Wine Initiative research programs under way in 2013, one of the 10 is about trunk diseases. A multi-crop (grape, pistachio and almond) project was funded in 2012 to tackle trunk diseases, Dr. Themis Michailides (UC Davis-Kearney in Parlier) and Dr. Kendra Baumgartner (ARS) pulled together a trans-disciplinary team to develop diagnostic tools and, eventually, measures to reduce the impact of diseases including *Eutypa* and *Botryosphaeria*. The research will cover wine grapes from five different regions and table grapes from two regions.
- Why is there not much written about trunk diseases in California now, in magazines such as ***PWV***, nor discussed at conferences, as occurs elsewhere in the world? I note that "red blotch" is to be discussed at the January 2014 Unified Symposium, but not trunk diseases.

However, the Lodi Winegrape Commission held a 2011 conference about trunk diseases, and another is scheduled. An outstanding presentation by Gubler and others is available on their website, with wonderful photos of symptoms. Lodi links are: lodigrowers.com/guide-to-managing-vineyard-trunk-disease-in-lodi/ and lodigrowers.com/eutypa-dieback-in-northern-california-vineyards-part-ii-of-ii.

- Maybe trunk diseases are not an issue in young vineyards anymore. Has "young vineyard decline" declined, and disappeared? Has concern for "red blotch virus" and "mealybug spread of leafroll virus" dominated disease thinking? Both are obviously serious issues, and worthy of attention. I believe grapevine trunk diseases are certainly more widespread. Concern raised by consultant Lucie Morton about "Black Goo" (Petri) disease in California in the 1990s led to the eventual formation of the International Council of Grapevine Trunk Diseases (see icgtd.ucr.edu), which held its eighth international meeting in Valencia in 2012.
- California growers do not think the trunk disease issue unimportant. A 2012 poll of AVF members found that trunk diseases were the third highest viticulture research topic, ahead of leafroll virus (fifth) and mealybugs (11th), from a total of 22 presented.
- I speak here of California, but the U.S. wine sector is more than California. What about other U.S. wine-producing states? I ask the same questions about health of young and old vineyards, and research and extension efforts.
- Somewhat like José Úrbez-Torres, I find trunk disease, especially *Botryosphaeria*, wherever I travel. This was most recently in Japan, on both young and old vines, and both *Botryosphaeria* and *Diaporthe eres* were found, the latter two on the island of Hokkaido. Again, the diseases were not previously described, and the death/decline was put down to old age or winter damage.

Might I have trunk disease in my vineyards?

American grape growers may well ask this question.

Let me list typical symptoms in mature and young vineyards, another column with photos is obviously required.

For mature vineyards, common symptoms are:

- Dead arms or whole vines. Often they were winter-pruned but fail to grow in the spring. They are often in patches, contiguous in vine and tractor row. These

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patches gradually grow. New symptoms can occur on isolated vines down-wind, spread by spores

- Trunk cankers and internal staining/dead tissue are common.
- Staining at the base of last year's spur may be seen.
- Sometimes early season shoot growth is inhibited, and in late season the vines may show mild chlorosis and apoplexy, the latter with wilting of shoots and fruit.

For newly planted vineyards, the symptoms are:

- Vine growth will be variable in the first year, and in general, some vines will fail to thrive. This variability will continue for the life of the vineyard, and the least vigorous vines will die first.
- Poorly performing vines, and even some healthy ones, may show internal staining. This may be black (indicating *Phaeomoniella*, *Phaeoachremonium* or *Cylindrocarpon*) or light brown (*Botryosphaeria*). The staining may be seen at the rootstock base, or under the graft union, or above the graft union.
- Some vines may not grow, others may fail in the first year, and some fail to grow during spring in following years. Depending on the degree of initial infection, "clumps" of infected vines may appear in subsequent years.

> Production practices have changed (including spur-pruning in various trellis systems), varieties have changed, chemical controls are different, according to **Paul Verdegaal**. "Arsenite took care of a lot of problems, as did copper, lime sulfur, parathion, weed oil, etc. Propagation methods have gone more to centralized nurseries.
>
> "During the last planting boom there was undoubtedly lots of poor quality (and/or infected) wood used for propagation to meet the demand. Nurseries are more careful now (maybe more care is needed?), and growers are more careful, but problems persist. Agricultural bankers like to see full production sooner rather than later. Legally or otherwise, plant material is moving faster and farther than ever before.
>
> "We do have a relatively dry climate in California, compared to many other areas of the U.S., and other more traditional districts in the world, but also areas such as Missouri, Arkansas, Pennsylvania, Michigan, Kentucky, even New York, etc. are only beginning to see local commercial *vinifera* vineyards and wine-growing districts 'mature,' and with that more problems.
>
> "We have limited chemical options right now with Rally, Topsin M, BrotoMax and VitiSeal. They cost money, require timely access; they and their application all come under regulatory scrutiny. Late pruning and/or cane pruning, with shoot thinning and reducing number and size of cuts if possible, along with protectants are standard suggestions. Variable weather patterns and the diverse array of pathogens make for a complex problem and don't make management easy or cheap."

Conclusion

My review of recent research presented here, my other reading and widespread vineyard observations suggest to me that the vine and wine world may be facing a new disease crisis, which can be compared to phylloxera in the late 19th century.

American readers may have a different perspective about phylloxera than vine growers elsewhere in the world. Why? Because phylloxera is native American, as are downy mildew and powdery mildew (Oidium). These two fungal diseases and the insect pest phylloxera almost brought European viticulture to its knees by the 1890s.

The situation for phylloxera was resolved eventually by grafting to native American vines, or their hybrids, as rootstocks. This is now a world-wide practice. The phylloxera problem was solved because a source of genetic resistance was found.

Now compare phylloxera and trunk diseases. Both are vine killers—in fact phylloxera the more quickly. Trunk diseases spread more slowly and are insidious; that is why they have escaped observation for so long.

I would guess that trunk disease is more widespread now than phylloxera. Remember that phylloxera is limited by quarantine in Australia, and most vineyards are own-rooted; Chilean vineyards are all own-rooted. So phylloxera is not everywhere, as in Washington state.

There is a cure for phylloxera which is cheap, environmentally friendly and sustainable: That is grafting to resistant rootstocks. There is no such cure for trunk diseases. There are no known sources of genetic resistance. We also have a situation where the majority of the world's nurseries are spreading trunk diseases; that is not the case for phylloxera.

These problems with trunk disease are not insurmountable. They can be overcome, as was phylloxera. The "cure" for trunk diseases will involve removal of infected vines, replanting with healthy material, and likely some pruning-wound treatment. Developing a cure will require increased recognition by growers and regulatory authorities and more research.

Final words about two paradoxical situations

One of my overseas clients is importing "high health" virus-free grafted vines from California. The photos (page 9) show trunk disease symptoms, but the California nurseryman assures my client there is "nothing to worry about." Humbug!

The second paradox also involves imported vines. Growers in the UK, where I now live, import "virus-free" but trunk disease-infected grafted vines that typically grow very poorly. The UK growers could much more cheaply propagate own-rooted vines, as they do not have phylloxera in their vineyards! But they are told (by the nurseryman's agents!) that they need rootstocks. Humbug!

I hope this column helps raise some awareness about trunk diseases. To solve the problem first requires recognition. I would be pleased to hear from others who might share my view, and may be concerned about the insidious spread of trunk diseases in North American vineyards. PWV

In writing this column, I acknowledge the excellent scientific work of Dr. José Úrbez-Torres and his Spanish colleagues David Gramaje and Josep Armengol. Were it not for Dr. Doug Gubler of UC Davis and his Ph.D. students, our knowledge of grapevine trunk diseases in the U.S. and contiguous countries would not be nearly so complete. Congratulations to all of you! I would also like to acknowledge the helpful reviews made on drafts of this column by Don Neel, Steve Thomas, Paul Verdegaal, Lucie Morton and José Úrbez-Torres. However, I take responsibility for the final text.

Dr. Richard Smart is rewriting and thoroughly revising "Sunlight into Wine." He is happy to receive statements from growers and winemakers about their commercial success with various aspects of canopy management. Interested persons should contact Dr. Smart by email at richard@smartvit.com.au. Dr. Smart visits the U.S. frequently, and consulting appointments also can be made by email. See smartvit.com.au.

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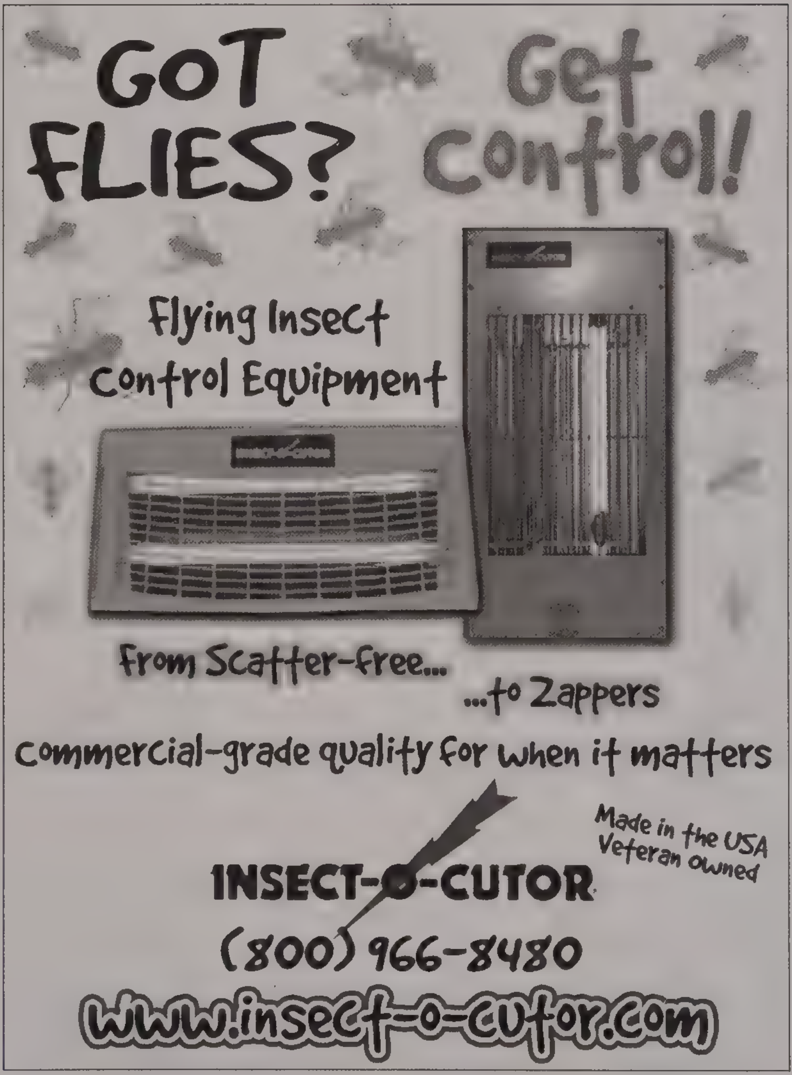


Ridge Monte Bello winery improvements

BY Don Neel, Editor

A custom-designed receiving hopper and a Vacher-Beguet Mistral sorting machine specifically for Bordeaux grape varieties were installed at Ridge Vineyards' Monte Bello winery in the Santa Cruz Mountains above Cupertino, Calif., in 2009. This began a five-year program of improvements, which includes additional tank capacity for the Bordeaux grapes, a new heating/cooling system with solar thermal collectors and a bio reactor for wastewater treatment completed in 2013.

The receiving hopper is 4 feet wide and 30 feet long with a slightly curved (to retain juice and grapes) poly conveyor belt with a variable-speed drive designed for easy cleaning and to achieve even, consistent delivery of grapes to the destemmer. Zinfandel is sorted for leaves and damaged fruit at the receiving conveyor.

Monte Bello Bordeaux grapes are destemmed but not crushed as whole berries go through the Mistral sorting machine at approximately 3 tons per hour. They are further sorted on a second conveyor by a crew of four to five. The Mistral sorting machine cannot be used for fully crushed Zinfandel grapes.

Tank room renovation included relocation of four 15-ton Zinfandel fermentors to be used for malolactic fermentations (MLF) and blending. Seven new 4.25-ton mobile primary fermentors were installed for the new replanting of additional 19th century blocks abandoned during Prohibition. A mezzanine was built above them to hold 12 580-gallon tanks to initiate natural MLF from the separate parcels of Bordeaux varieties.

Ridge fermentors are usually turned three times during harvest. Average Zinfandel fermentations typically run seven days before pressing. Zinfandel completes MLF in tank before transfer to barrels. Bordeaux varieties start MLF in tank and are transferred to barrel with malolactic lees to finish.

The tank sanitation procedure includes the use of a Lechler M20 tank cleaning head; first rinse with potassium hydroxide detergent and 150° F water 15 minutes for a 5-ton capacity tank. The second rinse is ambient water and citric acid for 15 minutes. The third rinse is 2–3 minutes ambient water.

There are nine acres of new Bordeaux variety plantings that have drip irrigation installed for use until the vines are sufficiently rooted.

Green retrofit of heating and cooling systems

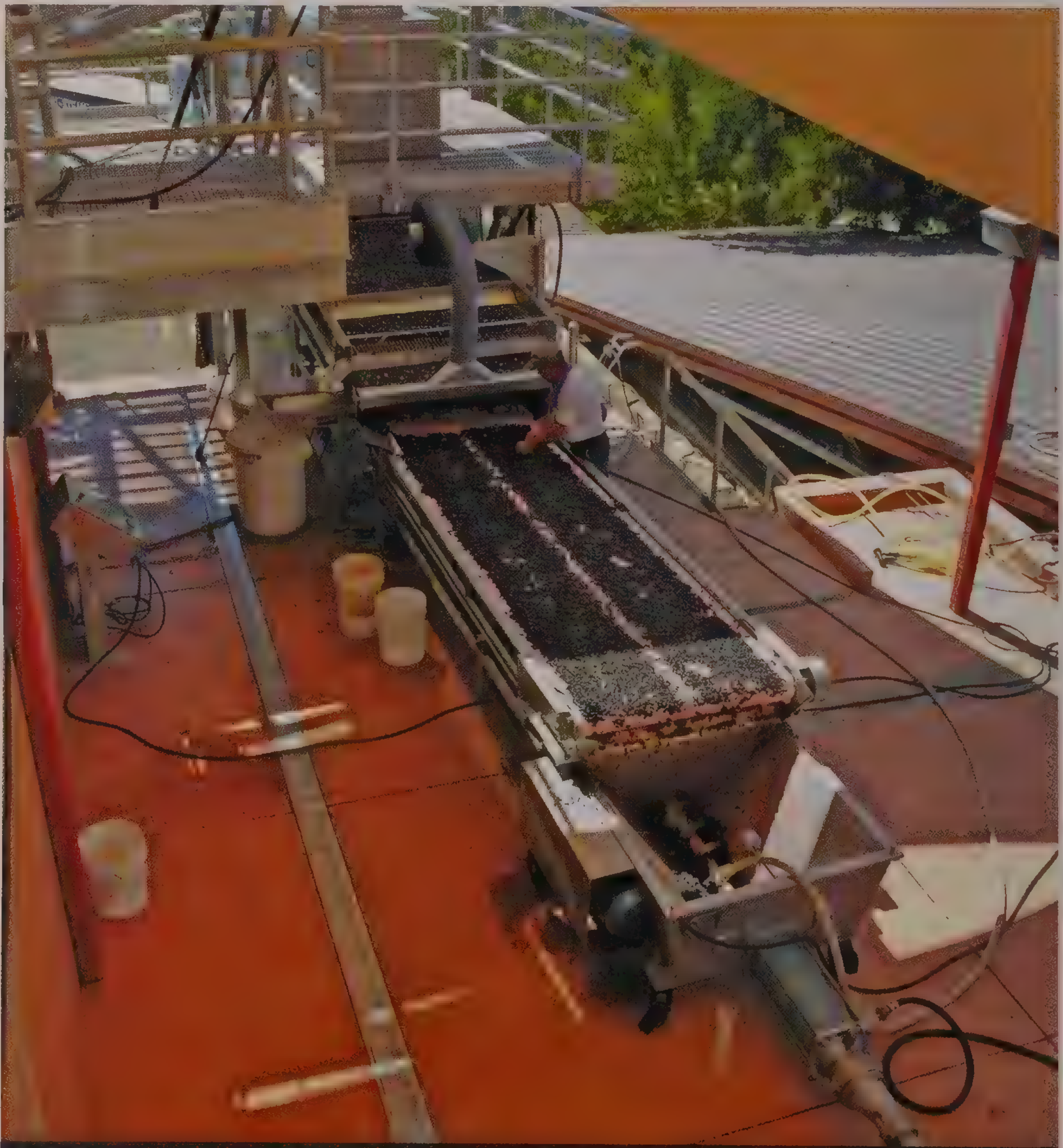
BY Zachary Domich, Solarix Systems, San Francisco, Calif.

Ridge Vineyards Monte Bello winery installed high energy-efficiency mechanical equipment for the 2013 harvest. Chief winemaker Paul Draper asked the owners of Solarix Systems in San Francisco, Calif., Zachary Domich and Bob Bullach, to design and install a system worthy of the Ridge brand.

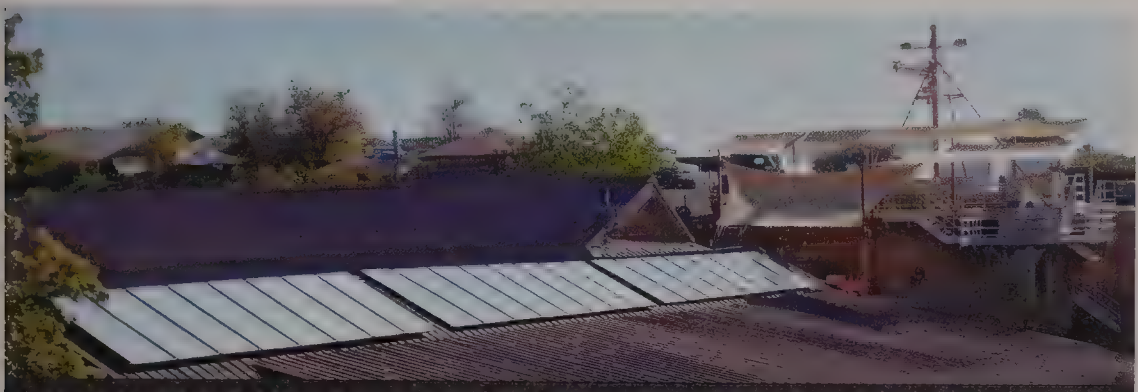
Through lengthy consultation with Ridge winemaker Eric Baugher, a system was implemented that most logically addresses the Ridge endeavor to make the best wine possible while utilizing the least amount of energy, without compromise.

With the history of Ridge Monte Bello dating back to the 1800s, its facilities have endured many revisions and upgrades. Ridge Monte Bello had outlived its most recent mechanical configuration, which was considered advanced when installed but after decades of service has become fatigued and outdated. The old system was decommissioned as the new system integration occurred.

As in many wineries, facility activity and dependence on the mechanical system continues year-round, leaving small windows of opportunity to execute time-consuming mechanical remodeling or upgrades without interrupting production. With the upgrade, the entire heating and cooling system was replaced, without interruption of harvest. Creativity was required in layout, configuration



A destemmer is positioned above the simple and effective Vacher-Beguet Mistral sorting machine that employs a slotted zone and an air knife to automatically remove unwanted stem pieces, shot berries, raisins, insects, and other MOG. A transfer conveyor feeds a KIESEL SP20FTF hopper-style progressive cavity must pump with a short, curvier, higher amplitude rotor that creates large pockets to move grapes gently through the pump with variable speed drive.



A south-facing roof of the winery adjacent to the grape-receiving pad (right) has 24 solar thermal collectors installed. When the sun is shining on the collectors, heat transfer fluid (water) circulates or is pumped through the collector field while absorbing heat. This thermal energy is stored and redistributed from an insulated 1,500-gallon storage tank inside the winery.

and execution.

To avoid downtime of any vital mechanical components during harvest season, Draper and Baugher allocated a portion of the facility previously used for about 50 wine barrels to house new mechanical equipment. This allowed them to design and build a complete new system while enjoying full functionality of the existing system.

The existing heating/cooling piping distribution network was deemed stable and reusable, therefore this extensive system remains in operation. New equipment was interfaced into the existing piping network while maintaining the ability to valve-in and -out new and old components as needed. This allowed for a seamless transition from the old to the new system, even during full harvest activity.

The hands-on approach to wine production at Ridge Monte Bello has relied on very little automation. Upgrading the outdated boilers, chillers and pumps, while incorporating solar thermal and chiller heat recovery required the integration of universally adaptable controls utilizing programmable logic (PLC).

In designing a system tailored to the needs of this facility, the energy-conservation strategy started with the integration of a new 30-ton chiller with heat-recovery capability. The chilled glycol from the evaporator side of the unit serves to feed fan coils for winery space-cooling, including the wine barrel rooms, offices, etc. This chilled glycol loop is also piped to allow for low-temperature wine tank cooling at most tank stations in the winery.

The by-product of the 30-ton chiller is hot water. The condenser side hot water is retained, stored and fed to five boilers in the form of pre-heated make-up water at temperatures as high as 140° F. A 1,500-gallon hot water storage tank serves as this heat-recovery storage. This tank is also heat-supplemented with 24 4-foot by 10-foot glazed flat plate solar thermal collectors that have the capacity to supply water temperatures as high as 180° F.

The configuration allows the 1,500-gallon "fossil fuel-free" thermal ballast to be distributed via heat exchangers to provide direct heating to all tank stations while remaining independent of gas-fired hot water. This energy-conserving functionality replaced the "gas-only" capabilities of the previous system and will reduce gas consumption consider-

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The system depicted in the photo is a bioPULSE™ MBR System installed at The RIDGE Montebello in Cupertino, CA

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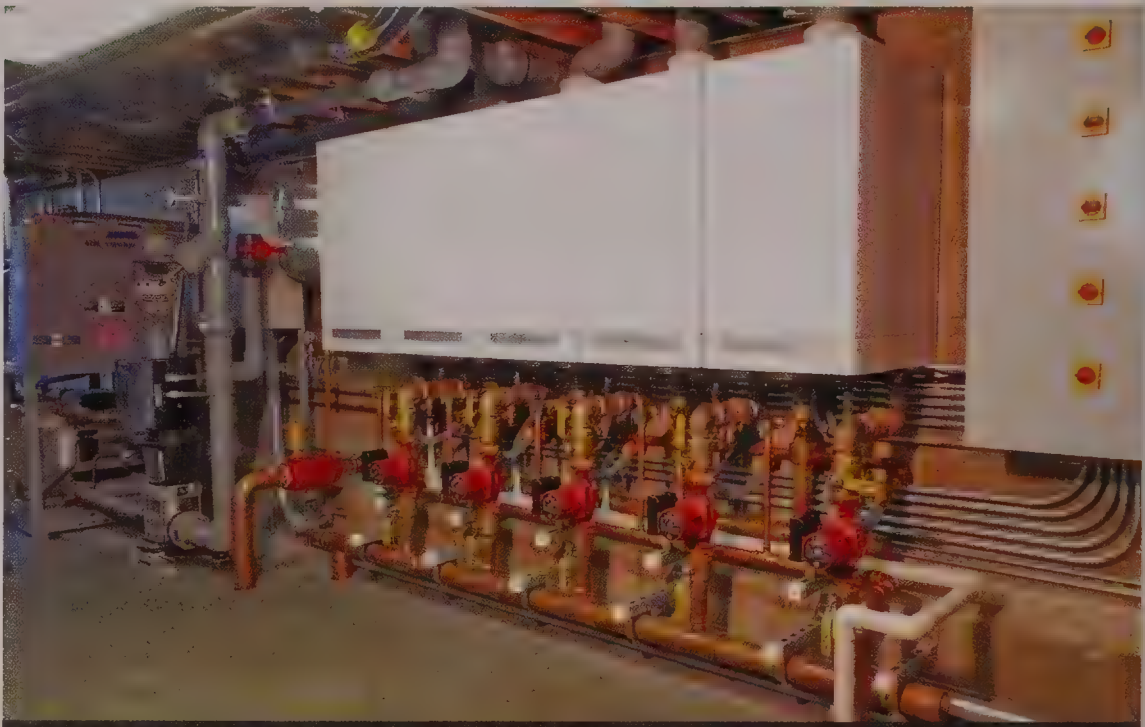

Five 370k BTU per hour boilers providing 96% efficiency, redundancy and versatility are mounted on a wall to conserve floor space. Heat recovery chiller (left) produces much needed hot water as a by-product.

ably. Features such as this required the universality of the PLC.

Five high-efficiency condensing boilers were installed. The five units have 96% efficiency along with modulating output and can allow for variation between 100,000 up to 1.776 million BTU per hour, only allowing 4% waste heat loss out of the flue.

Should the solar and chiller heat source become depleted, the five boilers (see photo at right) will sustain hot water supply in unison or separately, depending on demand, while the integrated controls rotate boiler duty-call, preventing uneven service hours between the units. Having five boilers also provides mechanical redundancy. Should any one of the boilers require service, the others will continue operation, preventing hot water downtime, which can be critical during harvest, especially in cold weather.

Electricty consumption is paramount when producing wine on a commercial level. Pumping hydronic solutions is a major electrical draw, therefore recent improvements in variable frequency drive (VFD) pump technology have proven key in this application.

In regards to older hydronic technology, the standard operating procedure was to size pumps for peak demand and not allow for a lower horsepower setting, should demand be less than maximum during off-peak periods. With variable-frequency-drive (VFD) pumps, the power consumption scales back as demand decreases, minimizing hydronic electrical consumption. With off-peak periods being a majority of the year, the annual electrical energy-savings will be substantial.

VFD pumps also allow for very accu-

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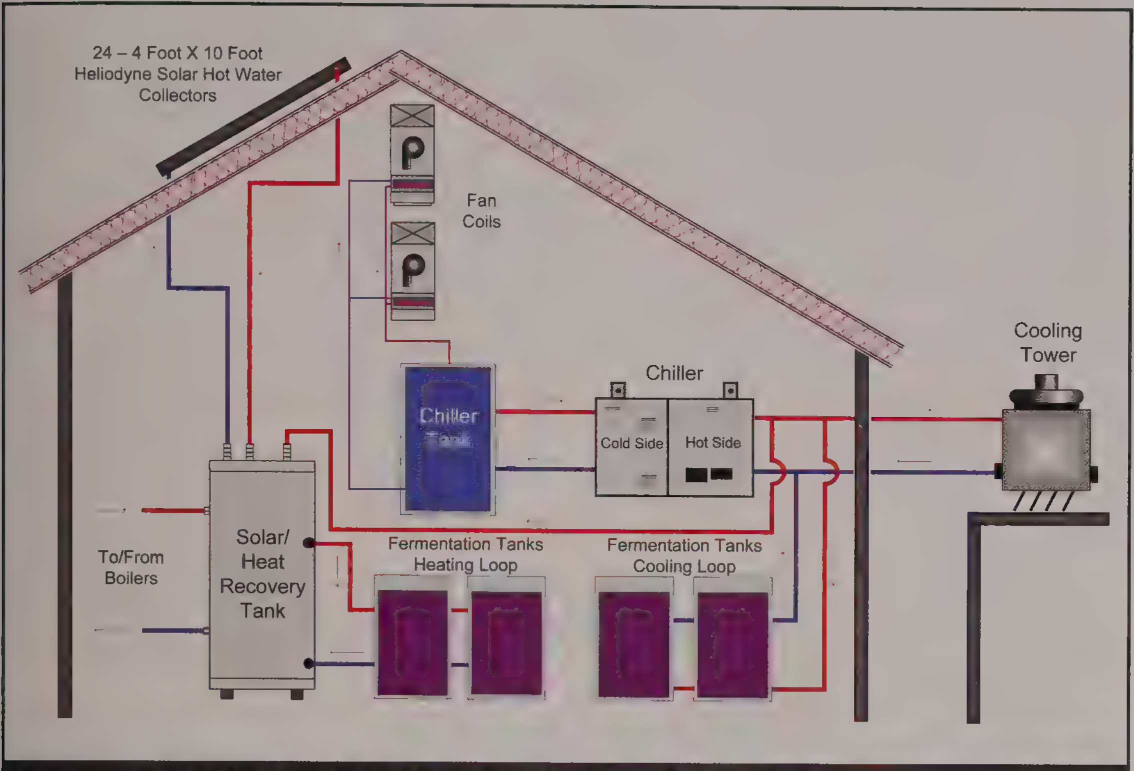
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Fermentation Tanks Cooling Loop

A representation of the new Ridge Monte Bello heating and cooling system.

rate temperature control. When fermentation tanks require heat, by varying the hydronic pump speed through the absorption side of the heat exchanger, we can dial the delivery temperature and GPM with precision and match the winemaker's thermal requests.

For example, Monte Bello winery receives most black grapes at an average of 62° to 64° F. After destemming and sorting of the grapes, and filling the fermentation tank, the tank jacket receives 78° F water that is circulated for 12 to 24 hours to raise the temperature of the grapes to 68° F and encourage natural fermentation to begin.

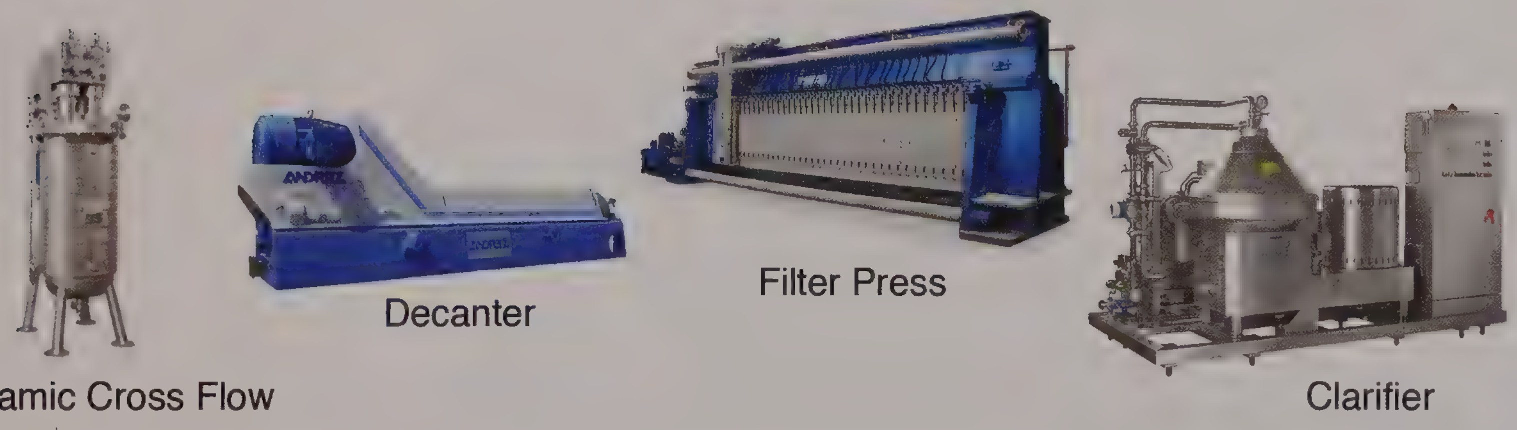
Having accurate and reliable hydronic control which mechanically conserves both natural gas and electricity while utilizing natural solar thermal and chiller heat recoverd energy was the goal.

Photovoltaics will be installed at the Monte Bello winery. As the electrical-metering reports are recorded and monitored, and the new Solarix System's reduction in electrical consumption is established over time, a full photovoltaic evaluation will be performed and the appropriate size system will be installed.

Wastewater purification for vineyard application

BY Peter Annunziato,
bioprocessH2O, Portsmouth, RI

Process wastewater generated by the Ridge Vineyards Monte Bello winery during crush and wine production is pumped to a Dontech Industries self-cleaning rotary drum fine screen (see page 18) that removes the solids, stems and particulate matter greater than 0.5



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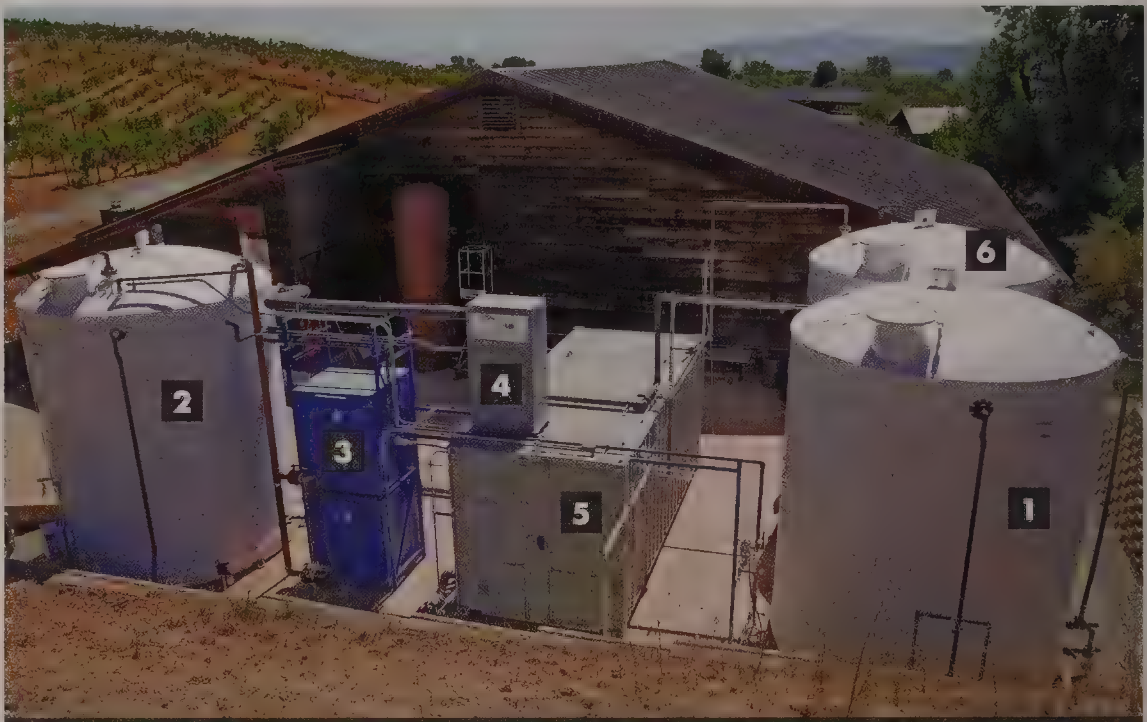

The bioprocessH2O system to treat wastewater includes an equalization tank [1], aeration tank [2], blowers [3], membrane hood [4], membrane skid, controls, chemical feed inside the shipping container [5], and sludge holding tank [6].

mm from the waste stream.

The screened wastewater flows by gravity into an integral sump where two multi-stage pumps transfer the screened wastewater to a 10,000-gallon equalization (EQ) tank prior to treatment in the membrane bioreactor (MBR) system supplied by bioprocessH2O.

The EQ tank provides surge and wastewater storage capacity to receive wastewater at a flow rate of 35 gallons per minute (70 GPM peak) from the fine screen transfer pumps. This allows a continuous wastewater feed of about 5 GPM into the 10,000-gallon aeration tank where the aerobic biological treatment occurs (oxidation of biochemical oxygen demand).

Wastewater in the aeration tank is biologically degraded by a population of heterotrophic bacteria and a diverse group of micro-organisms that consume the organic matter. The bacterial growth is regulated by the organic load entering the aeration tank and the amount of time the micro-organisms remain within the system.

Oxygen required for bacterial respiration is provided using positive displacement blowers, a fine bubble diffuser assembly and an automated dissolved oxygen (DO) control system. The control system measures the DO concentration within the aeration tank and responds by varying the amount of air supplied to the aeration tank using two variable speed drive (VSD) controlled blowers. The DO control system and VSD blowers are critical components to minimize power consumption.

Dontech Industries Inc. stainless steel externally fed rotary drum screen model RDS-WW-2512 WC is pictured with an oversized sump designed specifically for Ridge Winery to handle a peak flow of 75 gpm while removing solids 500 micron (0.02 inch) or greater from winery processing water. Overall dimensions are 44 inches x 44 inches x 88 inches tall with a 25-inch screen diameter. Collection drum inside yellow plastic housing receives solids that are transferred to winery composting operation.

A pH sensor and external controller provide for automatic dosing of potassium hydroxide to maintain the aeration tank pH between 6.5–8.0 while nutrients (ammonia and phosphorus) are also supplied to supplement the micro-organism's metabolic requirements.

Wastewater is pumped at a controlled rate from the aeration tank to a vertically configured bioPULSE™ Airlift tubular membrane. Diffused air is injected at the bottom of the membrane module to create an airlift pumping action and provide air scouring of the membrane surface to enhance and maintain system performance. This approach of utilizing air and liquid to provide membrane scouring results in an energy efficient MBR.

The semi-permeable polyvinylidene difluoride tubular ultrafiltration membranes have 5.2 mm diameter lumens with 0.03 micron pores passing clean water as ultrafiltration (UF) permeate. The bacteria and suspended solids commonly referred to as mixed-liquor suspended solids are rejected and returned to the aeration tank for multi-pass processing.

The UF permeate is pumped from the membrane module at a controlled rate using a VSD permeate transfer pump that is limited by a level sensor to maintain a constant liquid level within the aeration tank. The UF permeate is discharged to a distribution system for irrigation of grapevines and select trees in the vineyard.

The tubular membrane lumens are periodically back-pulsed by actuating a permeate backwash pump to reverse the UF permeate flow through the pores to dislodge solids, reduce membrane biofouling and sustain the permeate flow rate referred to as flux (gallons of permeate produced per square foot of membrane per day).

Additionally, the membrane lumens are typically cleaned once every four to eight weeks by a chemically enhanced backwash process. The entire process is automated using the PLC controller and monitored via a set of process viewing screens on an operator interface terminal.

The treatment process is automated using an Allen Bradley PLC and monitored via a set of process viewing screens on a color touch screen operator interface terminal. The treatment system can be controlled remotely through the PLC via an internet connection that allows bioprocessH2O to provide oversight and operational assistance to ensure successful system performance.

BioprocessH2O installed the UF membrane system, PLC control panel and chemical feed assemblies within a customized 20-foot long shipping container that facilitated system installation and ongoing operation and maintenance.

Biological sludge generated during the wastewater treatment process is digested on-site using an aerobic digestion tank that provides an environment in which the micro-organisms metabolize and consume a portion of the wasted biomass. PWV

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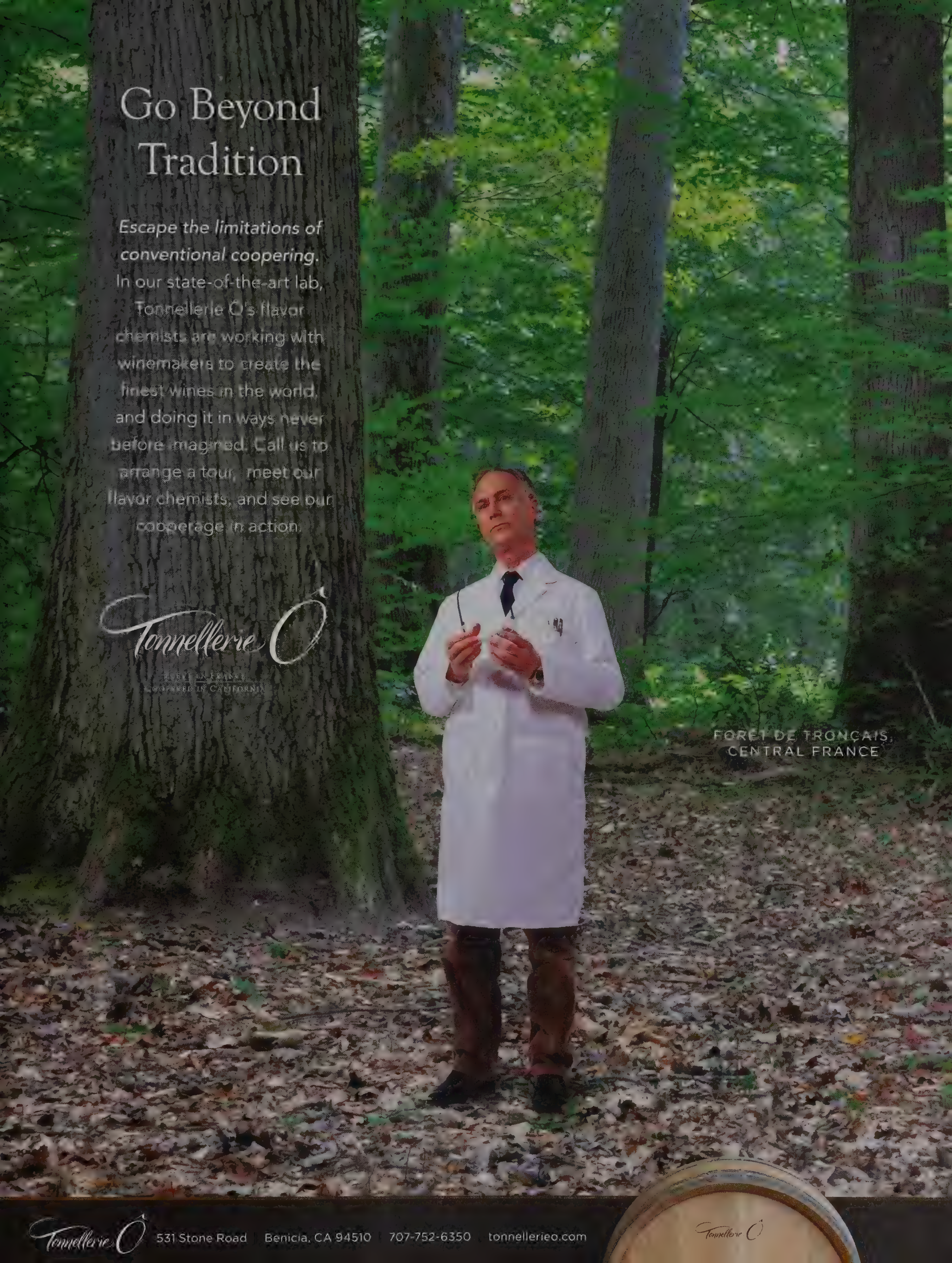
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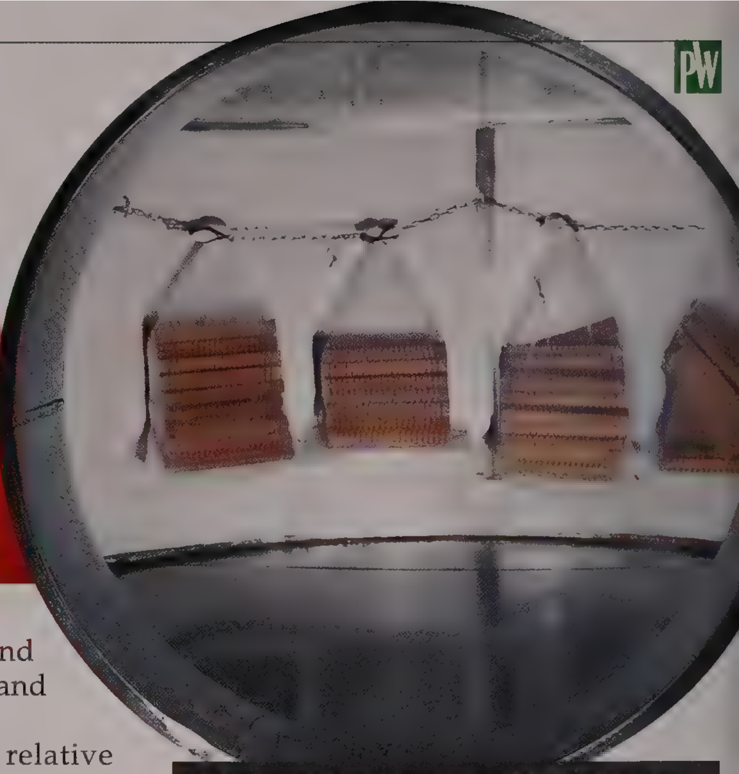
OAK INSERTS

The effect of grain orientation on extraction of oak flavor compounds



Nylon mesh bags containing 36 Fine Northern Oak cross-cut staves hang from a chain around the circumference inside a wine tank to allow oak flavor extraction into red wine.

BY Kevin Crawford, Almon Benton, Alyssa Dreger, Department of Chemistry, University of Wisconsin-Oshkosh; **David Plumb, Alfred Grigg,** Fine Northern Oak, Minneapolis, MN; Corresponding author email: crawfork@uwosh.edu

Properly aged and toasted oak, sourced from the proper species and growing climate, imparts desirable flavor characteristics to wine when used in the traditional fire-toasted barrels. The chemical composition of oak is variable depending on species as well as aging and toasting conditions.[1]

In modern winemaking, barrel alternatives provide a form that can be toasted in convection ovens, a reproducible method for imparting oak flavor compounds to a wine compared to expensive and relatively small-capacity barrels. Barrel alternatives also provide an opportunity for higher surface area exposure to the wine, resulting in more rapid extraction of oak flavor.

Extraction of flavor compounds from oak into wine is a time-dependent process. In a traditional 60-gallon barrel, the wine is exposed only to the parallel (vertical) grain of the oak (one side of the stave exposed to wine). All flavor extraction must occur from this surface. A wine might spend several months in barrel, and the barrel will still retain enough flavor compounds to be extracted during/from additional fills.

In contrast, barrel alternatives are submerged in the wine, providing exposure on both the parallel and cross-cut axes on all sides of the oak. Not only is more surface area exposed to the wine, but more importantly, two of these surfaces will consist of the cross-cut axis of the wood grain. When barrel alternatives are used, the entire piece of wood is exposed to the wine, allowing penetration and extraction along the cross-cut and parallel axes.

This text edited and expanded by the authors from first publication: November/December 2010.

In order to determine the relative extraction rates of oak flavor compounds based on grain orientation, we prepared blocks of wood with epoxy coating, preventing wine exposure on either the cross-cut or parallel axis. These blocks were soaked in a model wine solution that was sampled weekly for SPME-GC-MS quantification of furfural and vanillin (first experiment) or more frequently sampled and analyzed by HPLC for a wider variety of molecules (second experiment).

Our goal was to determine the relative contribution of cross-cut grain exposure to the rate of extraction of furfural and vanillin from toasted oak.

Our results indicate that furfural is rapidly extracted from the cross-cut axis relative to the parallel axis and that vanillin extraction was only measurable in the cross-cut axis exposed wood during the 12-week exposure (first experiment).

Our second experiment demonstrates that cross-cut grain exposure in oak impacts both rate of extraction and total extractable amount of oak flavor molecules. As expected, access to the natural pores of the wood increases the rate of extraction of all molecules studied.

More significantly, molecules with limited water solubility and larger size were extracted in higher amounts from cross-cut oak. These results demonstrate that maximum exposure of the cross-cut axis of oak pieces will result in a much more rapid extraction of flavor compounds into wine.

Solid Phase MicroExtraction–Gas Chromatography–Mass Spectrometry (SPME-GC-MS) is a preferred method for quantifying desirable and undesirable flavor and odor compounds in wine.[2,3,4] This method extracts and concentrates the flavor compounds (SPME) followed by separation (GC) and identification/quantification of the compounds (MS). It also involves much less manual sample preparation than the traditional liquid-liquid extraction and concentration techniques.

High-Performance Liquid Chromatography (HPLC) is an alternative to gas chromatography that is suitable for molecules that may break down in a gas chromatography (high-temperature) system. This includes polar molecules such as gallic and vanillic acids. The method relies on a liquid phase to conduct the separation with detection of the molecules based on their ability to absorb ultraviolet light. Minimal sample preparation is required, but it cannot detect molecules at the same low levels as SPME-GC-MS.

Vanillin and furfural are two desirable flavor compounds present in toasted oak (see structures in Figure 1a on page 22.) Furfural is the smaller of the two compounds and is more volatile than most other flavor compounds present in oak. It also has a low octanol-water partition coefficient (log P) indicating high water solubility. Furfural and similar compounds are responsible for a sweet and butterscotch flavor in wine.

Vanillin has a higher molecular weight and is less volatile than furfural and many of the other flavor compounds extracted from oak. It also has a much larger log P value, indicating less water solubility.

Oak inserts added to wine tanks at Fetzer Vineyards

"We have used Fine Northern Oak inserts (medium toast) in Cabernet Sauvignon and Merlot for more than six years," says Mike Chupp, Fetzer Vineyards red winemaker in Hopland, Calif. "Inserts remain in tanks ranging from 15,000 to 30,000-gallon capacity between four and eight months—the average is six months.

"We anchor nylon mesh bags containing 36 12.5-inch long inserts each to a chain about 3 feet above the tank floor, and then fill the tank with wine, allowing the bags of inserts to float in the bottom quarter of the tank. 250 inserts are used in a 15,000-gallon tank. The tank full of wine is sparged with nitrogen monthly to mix the tank. Micro-ox is also occurring while the FNO insert bags are in the tank. The wine temperature is between 58° and 65° F.

"A tank of wine aged with FNO bags of inserts plus micro-ox may end up to be about 10% of a bottling blend," concludes Chupp.

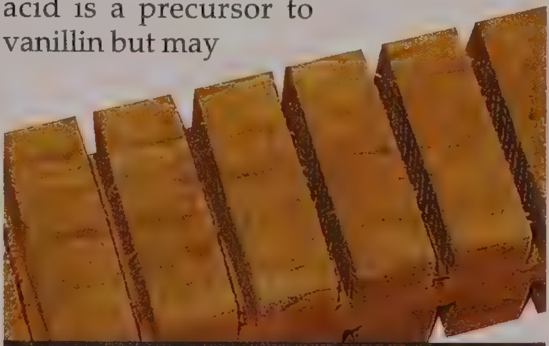
Fetzer Vineyards uses nylon-mesh bags containing barrel alternatives in tank ageing of red wine including: Fine Northern Oak cross-cut staves, Innerstave Fan Systems, Nadalie Oak Add Ins, Oak Solutions Group's evOAK tank staves, and Stavin Stave Segments. "We continually evaluate barrel alternatives to determine what will work best for Fetzer consistency with the style and quality of our winemaking," says Dennis Martin, director of winemaking.

"FNO gives us the toasty, sweet oak spice components that enhance the flavor and aroma characteristics of Fetzer wine blends and complements our style," adds Martin.

"We use approximately 60% French oak and 40% American oak in the Cabernet and Merlot," reports Chupp. "Zinfandel is about 60% American and 40% French. We use predominantly medium toast but some med+ is also in the mix."

Other researchers have shown that these compounds extract at different rates due to their different properties. Idoia Jarauta observed that furfural appeared to reach a maximum level in barrel-aged wine after only six months while the vanillin concentration continued to increase through 24 months.[5] Philip Spillman detected increasing vanillin through 23 months in barrel-aged red and white wines.[6]

Gallic acid is a phenolic antioxidant[7] that can be converted to tannic acids that impart astringency to the wine. Vanillic acid is a precursor to vanillin but may also be biologically transformed to guaiacol by certain bacteria.[8] Syringaldehyde is similar in structure to vanillin and contributes to the overall vanilla flavor in wines. The structures and log P values for these compounds are shown in Figure 1b.



This cross-cut stave insert is 12.5 inches long, with notches 1/2-inch wide and 1-inch deep. The surface area created by notches increase rate of wine penetration, accelerating extraction of flavor compounds.

The format of the oak alternative can also impact the rate of extraction of flavor

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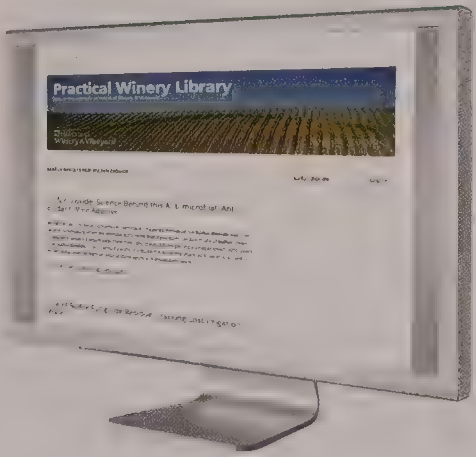

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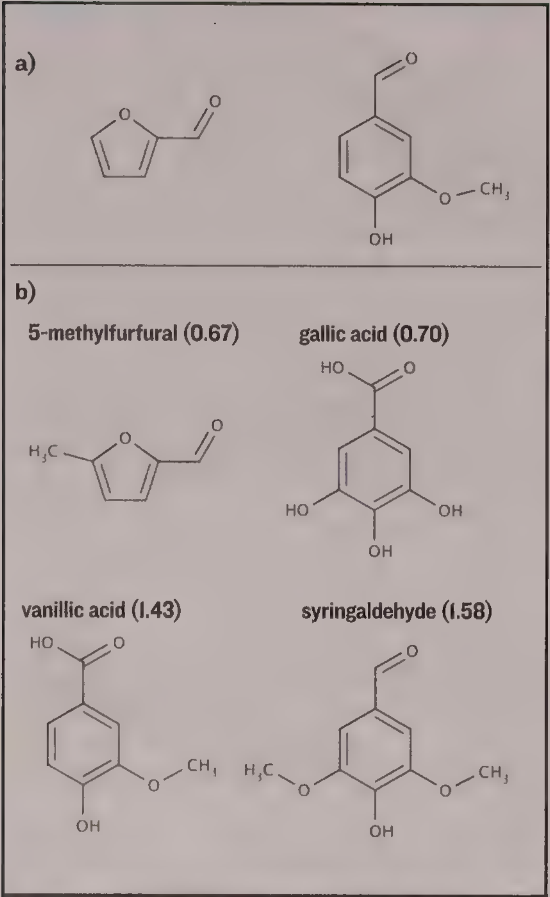


Figure 1: a) Structures of furfural (0.46), and vanillin (1.21). b) 5-methylfurfural (0.67), gallic acid (0.70), vanillic acid (1.43), and syringaldehyde (1.58).

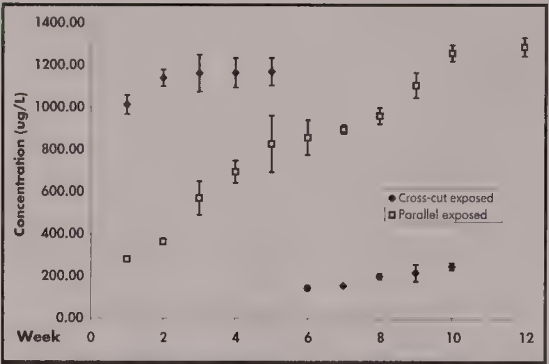


Figure 2: Time course of furfural extraction from cross-cut and parallel-exposed wood (Experiment 1). The cross-cut exposure extraction solution was changed after removing the fifth sample.

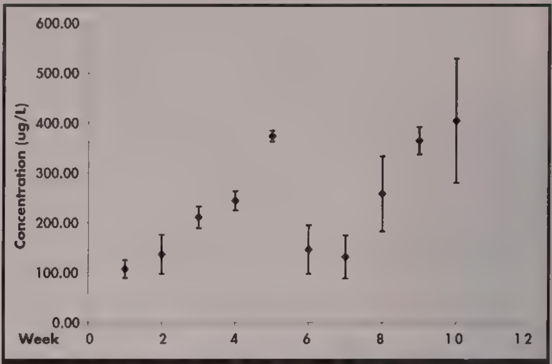


Figure 3: Time course of vanillin extraction from cross-cut exposed wood (Experiment 1). Extraction solution was changed after removing the fifth sample.

compounds. Marta Cano-Lopez demonstrated that tasters could detect differences in finished wine exposed to different formats of barrel alternatives such as powder, shavings and cubes, with the effect being most pronounced after six months, indicating a different rate of release of flavor compounds from each barrel alternative format.[9]

Our goal was to determine the relative contribution of cross-cut grain exposure to the rate of extraction of flavor molecules from toasted oak. By applying epoxy to seal one type of grain (cross-cut or parallel) on toasted oak cubes, we were able to separately measure extraction from the cross-cut axis and the parallel axis of oak cubes.

Two different experiments were conducted: The first on a smaller scale with equivalent masses of oak and measurement of furfural and vanillin by SPME-GC-MS. The second experiment used equivalent masses and surface areas of oak (but different grain exposures), larger sample volume, more frequent sampling and monitoring of a larger number of molecules by HPLC in order to measure

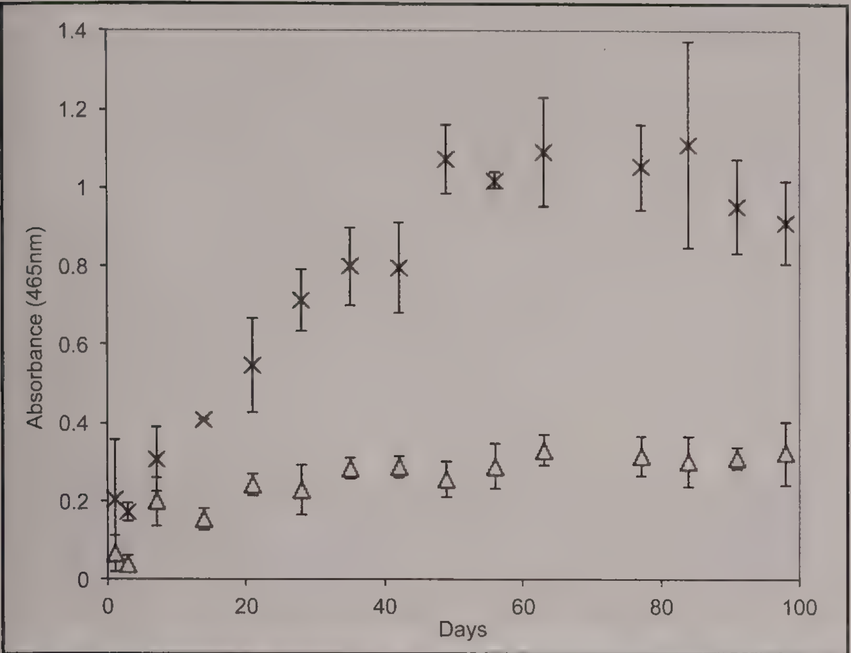


Figure 4: Time course of optical absorbance of model wine solution (465nm) during oak extraction (Experiment 2). Δ = absorbance of the solution exposed to parallel-grain oak, X = absorbance of the solution exposed to cross-cut oak.

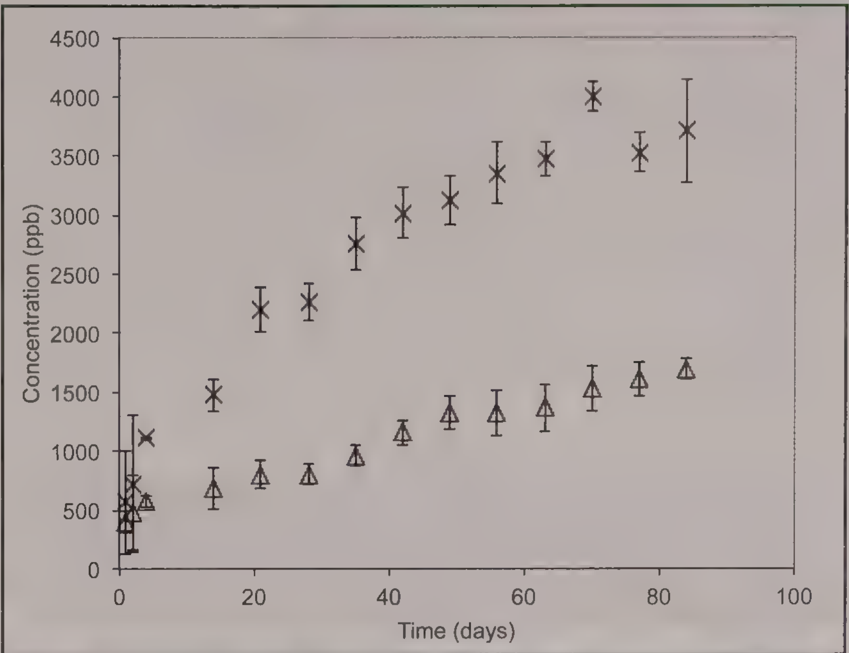


Figure 6: Concentration of gallic acid to time (Experiment 2). Δ = concentrations in solution exposed to parallel-grain oak, X = cross-cut-exposed oak solutions.

rates of extraction and final concentrations for a wider variety of molecules.

Materials and methods

Oak samples: Tight-grain American white oak *(Quercus alba)* heartwood was obtained from the Staggemeyer Stave Mill (Caledonia, Minn.). The oak was quartersawn to the conventional 1.25-inch-thick barrel stave and seasoned for two years.

Sample preparation: Oak staves were toasted throughout using an extended toast protocol, then cut into cubes (1.25 x 1.25 x 4.0 cm for Experiment 1 or 1.90 x 1.50 x 8.0 cm for Experiment 2). Cross-cut exposed cubes were covered with epoxy on each parallel axis (four sides), while parallel-exposed cubes were covered with epoxy on each cross-cut axis (two sides).

The resultant surface area exposed was 40 cm^2 for the cross-cut exposed sample and 52 cm^2 for the parallel-exposed sample (Experiment 1) and 30 cm^2 per block for both grain exposures (Experiment 2). The cubes were weighed before the epoxy was applied to ensure an equal mass of oak was used in each extraction.

Model wine preparation and oak extraction: Model wine solutions were chosen for this study to eliminate the removal of oak flavor molecules due to biological actions and reactions with red wine pigments.

Experiment 1: Two extraction flasks were prepared with 13.26 g of oak (one with all cross-cut exposed pieces, the other with all parallel-exposed pieces) in each flask and 1,326 mL of 13% ethanol in deionized water solution. (Note that 10 g/L of extracted oak flavor is more than used in typical winemaking blends. Usually a tank with such a concentration is blended with unoaked wine to achieve the wine's intended style for bottling.)

After each week, five 10-mL aliquots were removed from each flask and prepared for SPME-GC-MS analysis. The cross-cut exposed sample solution reached a maximum furfural concentration after two weeks; the solution was removed and replaced with a fresh extraction solution after the week-five sample was collected.

Experiment 2: Six different 3-gallon (10.14 L) glass jugs were filled by mixing 1.32 L of 95% ethanol in 8.68 L of deionized water, and 70 g (7 g/L concentration) of tartaric acid (for pH control) was added to each. A 50% sodium hydroxide solution was added drop-wise to the mixture to adjust the pH to 3.3.

Six cubes of cross-cut-exposed oak samples were placed in each of three vessels, while six cubes of the parallel-exposed oak samples were placed in each of the other three vessels for a total mass of 89 g and surface area of 182 cm^2 per container. Three 10 mL aliquots were removed from each sample on days one, three and seven, and then each consecutive week for 14 weeks. Each sample was tested for color by measuring its absorbance at 465 nm as an indicator of extracted phenolics.

Quantification: In Experiment 1, furfural was quantified by SPME-GC-MS against an internal standard with comparison to a calibration curve. Vanillin was quantified in the same way using a second internal standard for comparison. In our method, the lower limit of detection (LOD) of furfural and vanillin was 100 µg/L.

HPLC quantitative analysis (experiment 2) was performed using the absorbance at 280 nm for furfural, 5-methylfurfural, vanillin, vanillic acid and gallic acid. Syringaldehyde was quantified using its absorbance at 310 nm. Retention times of known standards were compared to the extracts to determine identities of the peaks. External standard calibration was conducted using known standards dissolved in 13% ethanol/water solution.

Results

Furfural (Experiment 1)

Figure 2 is a graph of the concentration of furfural in the ethanol/wine mixture over time for both parallel-exposed and cross-cut exposed samples. The concentration of the solution containing the cross-cut exposed cubes reached a maximum after only two weeks of exposure (1,139±40 µg/L). The extraction solution was then removed after week 5 in order to test whether additional furfural would be extracted. The graph demonstrates that the furfural concentration did not grow to the same level as the first extraction, achieving only 245±17 µg/L after an additional five weeks of extraction.

The parallel-exposed sample solution however continued to increase in furfural concentration over the entire 10-week period. Because it was not clear if the concentration had reached a maximum level, one additional set of aliquots was withdrawn from the cross-cut exposed flask after the 12th week (included on the graph). This sample showed a concentra-

Compound	Longitudinal Exposure Extraction Rate (r^2 value)	Radial Exposure Extraction Rate (r^2 value)
Furfural	312 (0.91)	189 (0.99)
5-methylfurfural	58 (0.97)	25 (0.99)
Vanillin	28 (0.97)	12 (0.79)
Syringaldehyde	89 (0.97)	34 (0.76)
Vanillic Acid	91 (0.97)	34 (0.95)
Gallic Acid	73 (0.95)	18 (0.93)

Table I: Initial extraction rates for compounds measured by HPLC during extraction based on slope of concentration to time graph over the first 21 days of extraction. Units are μg/L-day.

Compound	Longitudinal Exposure Final Concentration	Radial Exposure Final Concentration	p-value
Furfural	13.0±0.3 ppm	12.0±0.9 ppm	0.9
5-methylfurfural	2.10±0.07 ppm	1.61±0.1 ppm	0.5
Vanillin	1.22±0.10 ppm	0.69±0.15 ppm	0.05*
Syringaldehyde	4.7±0.3 ppm	2.2±0.4 ppm	0.0008*
Vanillic Acid	4.0±0.5 ppm	1.8±0.4 ppm	0.002*
Gallic Acid	3.8±0.6 ppm	1.4±0.3 ppm	0.001*
Guaiacol	49±9 ppb	33±1 ppb	0.04*
4-methylguaiacol	17±2 ppb	8±2 ppb	0.01*
4-ethylguaiacol	85±7 ppb	51±6 ppb	0.003*
Eugenol	10.2±0.7 ppb	5.8±0.4 ppb	0.0008*

Table II: Summary of final concentrations of flavor molecules in model wine solution. Each number represents the average of three model wine solutions. Significant differences are indicated with a * (p-value 0.05 or less).

tion very similar to the 10-week sample (1,257±38 μg/L at 10 weeks, 1,286±44 μg/L at 12 weeks).

The results demonstrate that furfural is completely extracted from cross-cut exposed oak in approximately two weeks, while more than 10 weeks is required for extraction of furfural from parallel-exposed oak. This result is significant considering that the relative surface area of the parallel-exposed oak is greater than the cross-cut exposed oak (52 cm² compared to 40 cm²). We therefore conclude that the extraction of furfural from the cross-cut axis of oak occurs at a rate at least five times greater than extraction from the parallel axis.

Vanillin (Experiment 1)

Figure 3 is a graph of the concentration of vanillin in the extraction solution in a 10-week experiment. Only the cross-cut exposed sample is plotted as the parallel-exposed flask had no measurable vanillin (less than 100 μg/L) during the entire experiment.

The cross-cut exposed sample showed an increasing vanillin concentration through the first five weeks, when the extraction solution was changed (due to the complete extraction of furfural as mentioned above). The vanillin concentration did rebound to approximately the same level in the second extraction solution as it reached in the first. The slope of the vanillin graph is similar in both five-week extractions. The vanillin results demonstrate that this compound is extracted much more readily from cross-cut exposed oak than from parallel-exposed oak.

Comparison of furfural and vanillin (Experiment 1)

It is clear that furfural extracted more rapidly than vanillin from both cross-cut exposed and parallel-exposed wood. Furfural is both a smaller molecule and more water-soluble than vanillin, which could explain some of the difference in extraction rates between these two compounds rather than any differences in distribution through the wood.

It is important to note that furfural extraction from cross-cut exposed wood was complete after about two weeks, while vanillin continued increasing through the fifth week and was also extracted into the second solution. This effect indicates that re-use of oak alternatives would result in different flavor profiles due to the differences in extraction rates for these two compounds.

For example, a five-week exposure of the cross-cut exposed oak to a wine solution would result in about 1,200 μg/L of furfural and almost 400 μg/L of vanillin in wine after the first use. Upon re-use of the oak, a five-week exposure to fresh wine would result in only minimal furfural (less than 300 μg/L) and about 400 μg/L of vanillin.

The results also demonstrate that vanillin is extracted much more efficiently in cross-cut exposed wood than parallel-exposed wood. This result demonstrates the benefit of cross-cut grain exposure in barrel alternatives. In our study, the cross-cut exposure provides a much more rapid extraction of the measured flavor compounds into the wine compared to parallel exposure.

Experiment 2

After one week the model wine solutions containing the cross-cut exposed oak had a noticeably darker appearance than the solutions containing the parallel-exposed oak indicating a higher level of extracted phenolics. Both the visible absorbance at 465 nm and the six HPLC-measured analyte compound concentrations were higher in the model wine solution containing longitudinal-exposed oak after only one day of extraction.

Figure 4 is a plot of the absorbance of the model wine solution at 465 nm versus time of extraction. The average absorbance for each of the three replicates is plotted with its standard deviation. Both solutions appear to reach a steady-state absorbance well before the end of the 14-week extraction period. The absorbance of the model wine containing cross-cut exposed oak was more than three times that of the solution containing parallel-exposed oak.

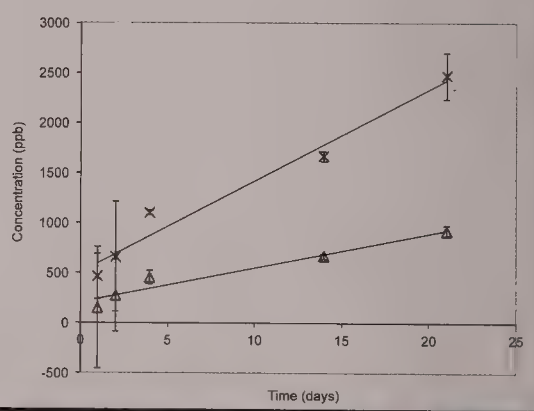


Figure 5: Concentration of vanillic acid to time for the first 21 days of extraction (Experiment 2). Δ = concentrations in solution exposed to parallel-grain oak, X = cross-cut-exposed oak solutions. Slope of cross-cut data = 91μg/L-day, slope of parallel data = 34μg/L-day.

Rate of extraction (Experiment 2)

In order to determine the rate of extraction of the analyte compounds, the slope of the concentration versus time graph was measured using the first five data points (day one, three, seven, 14 and 21) for each analyte determined by HPLC. Figure 5 is one example of the data used for this analysis, showing the concentration of vanillic acid related to time for each type of oak grain exposure.

All six compounds showed a much higher rate of extraction for the cross-cut exposed oak compared to parallel-exposed oak. The slope results and correlation coefficients are presented in Table I and demonstrate that all compounds are extracted from the cross-cut exposed oak at a faster rate than the same compounds from parallel-exposed oak.

Cross-cut exposure of oak likely provides more rapid access of the model wine solution to flavor molecules in the wood through natural pores in the wood. This is demonstrated by the higher rate of extraction for all six molecules measured by HPLC and the higher slope of the absorbance at 465nm. As the samples were controlled for mass, surface area and toast level, the only difference remaining between them was grain exposure.

Final composition (Experiment 2)

The final concentration of each analyte is presented in Table II. These numbers are the average of the final concentrations from all three extract solutions for each grain orientation. Figure 6 presents the concentration data for gallic acid in the 14-week period to demonstrate that the concentration of the analytes has reached a static level for both parallel and cross-cut-exposed oak solutions. This result was observed for all analytes and the absorbance of the solution.

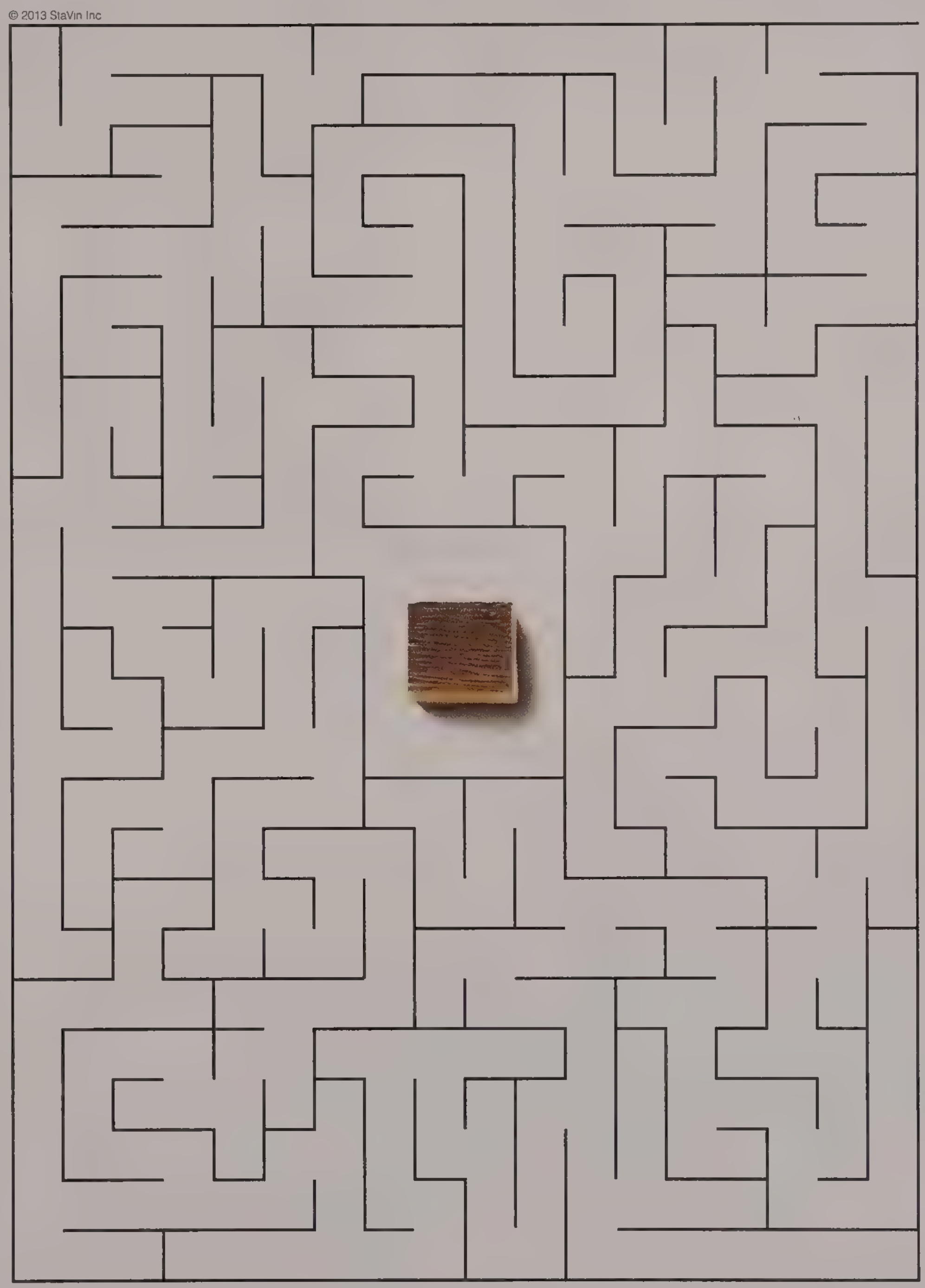
Since replicate extractions were conducted, we were able to perform a statistical comparison of the final concentrations. These results (Table II) show that furfural and 5-methylfurfural concentrations are nearly identical between both types of oak grain exposures. However, the other compounds are at significantly higher concentrations for the cross-cut exposed oak samples. Furfural and 5-methylfurfural have the smallest octanol-water partition coefficients of the compounds in this study (log K_{ow} of 0.41 and 0.67 respectively), though gallic acid has a log K_{ow} of 0.70, close to that of 5-methylfurfural.

The fact that the model wine solution has faster access to the oak through the wood pores in a cross-cut grain exposure implies a faster rate of extraction but does not suggest a different final concentration given enough time for extraction. However, in spite of controlling for mass and surface area of the oak we found higher final concentrations of most analytes extracted from the cross-cut exposed oak samples.

Figure 6 demonstrates that the parallel-exposed oak solution reached a maximum gallic acid concentration at about 10 weeks, indicating that all the available gallic acid had been extracted and additional extraction time would not have resulted in a higher final concentration.

The other analytes showed a similar result, reaching a maximum concentration well before the end of the 14-week extraction for both cross-cut and parallel-exposed oak samples. The only two molecules that did not have significantly different final concentrations had the smallest molecular weights and the highest water solubilities.

These results demonstrate that extraction of larger flavor molecules with lower water solubility can only be maximized by exposure of the cross-cut grain of the oak. In essence, with only parallel grain exposure a larger percentage of the flavor molecules in the oak will not be extracted at any time. This suggests that to maximize flavor from oak and minimize the amount of oak to be used to flavor wine, a cross-cut grain orientation must be used in addition to or exclusive of parallel grain exposure.



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Conclusion

Cross-cut grain exposure greatly impacts the rate of extraction of flavor compounds from toasted oak and the total amount of extractable flavor compounds. Additionally, the rate of extraction of smaller, more water-soluble molecules is much greater than that of larger less water-soluble molecules. Furfural was completely extracted from the cross-cut exposed oak within three weeks, thus reuse of such barrel alternatives would result in different flavor compositions in finished wine.

In order to maximize the amount of flavor molecules extracted from oak and minimize the amount of oak required to flavor wine, the wood should have some cross-cut grain exposed to the wine solution. PWV

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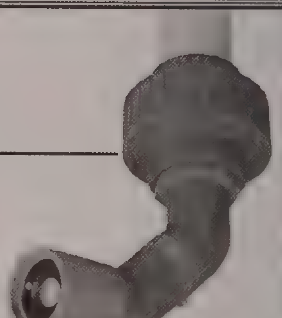


Manipulating light in the fruit zone improves WINE QUALITY

BY Katja Šuklje[1], Nico Gobler[2,3], Zelmari Coetzee[3], Klemen Lisjak[1] and Alain Deloire[3,4*]

Modified light and temperature in the fruit zone can have a positive role on the cluster microclimate, which consequently affect berry composition and therefore may improve wine quality or style.

However, it is important to remember the following:

- The relationships between fruit composition and wine quality or style are not direct. In other words, fruit analyses may give results on the concentration of a specific compound which will not reflect in the wine composition or in wine sensory analysis. Wine is a complex matrix within which numerous chemical compounds are interacting together and in addition, aromatic precursors present in the fruit are transformed during fermentation.
- The positive role of increased light on fruit composition has been well described by numerous authors. It should be noted that this abiotic factor has to be managed very carefully to avoid damage to the fruit (sunburn, berry shriveling, etc.), negatively affecting the fruit composition. In this regard, the thresholds of light and temperature's effect on the fruit composition are not well known and need further investigations.
- Fruit and canopy microclimates have to be managed to respect a specific site, canopy architecture and row orientation.

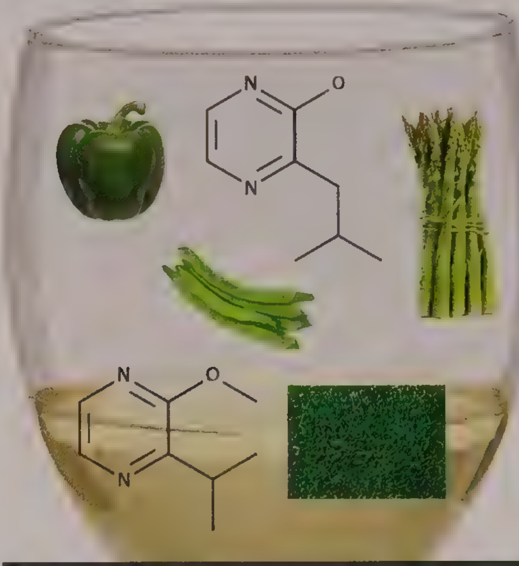


Figure 1: Attributes commonly used to describe methoxypyrazines present in Sauvignon Blanc wine: green pepper, asparagus and green beans.

To demonstrate the positive role of light and temperature at the fruit level and to show the complexity of the relationships between fruit composition and wine style (wine composition and wine sensory description by a trained panel), green/herbaceous and tropical flavors have been chosen as sensory attributes for Sauvignon Blanc.

South Africa offers a great variety of Sauvignon Blanc wine styles, ranging from tropical to greener, herbaceous to the diversity of the climate and soil of the Western Cape region. The greenness in Sauvignon Blanc could be considered a wine style up to the point where the green attributes dominate, resulting in a one-dimensional wine. In red wines, mainly Merlot and Cabernet Sauvignon, the perception of green attributes may be considered an undesirable aroma.[3]

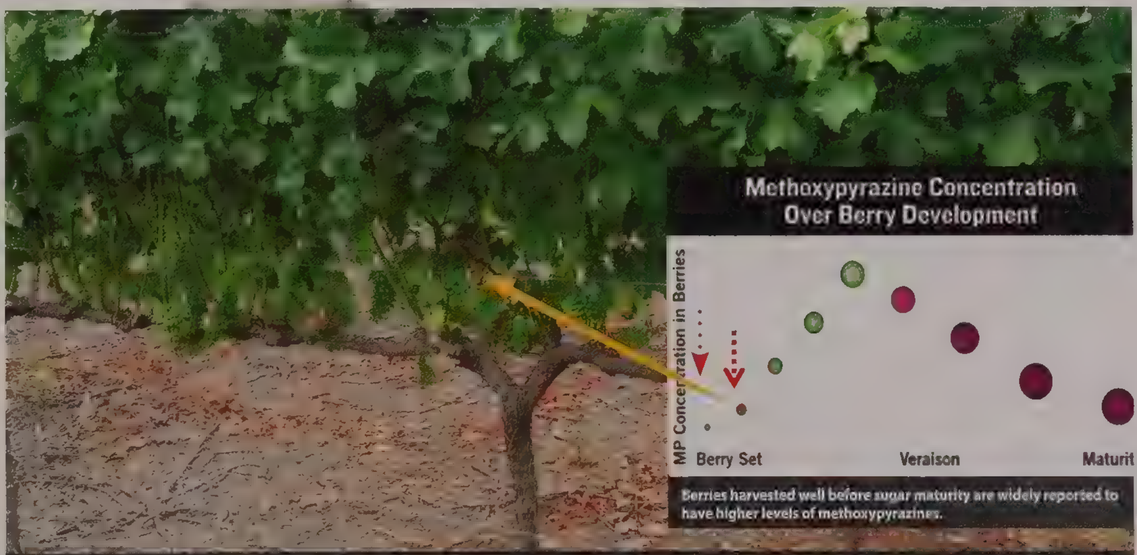
The green aroma descriptors of Sauvignon Blanc, Merlot, Cabernet Sauvignon, Cabernet Franc and Carmenere grapes and wines originate from 3-alkyl-2-methoxypyrazines (MPs). The most important MP found in grapes and wine is 3-isobutyl-2-methoxypyrazine (IBMP), whereas 3-isopropyl-2-methoxypyrazine (IPMP) is rarely present in grapes at harvest.

IBMP contributes to the green pepper, pea, and asparagus aromas while IPMP imparts earthier aromas.[1,11] The sensory detection threshold for IBMP was found to be very low; 2 ng/L in water and white wines and around 15 ng/L in red Bordeaux wines.[1,14]

In some Merlot and Cabernet Sauvignon wines, aromatic attributes related to eucalyptus and mint are found, which results in the wine sometimes being described as green. These attributes are, in fact, not linked to IBMP, although further research is needed.

IBMP in the grape berry is located in the skins (95%), seeds (4%) and pulp (1%).[14] IBMP biosynthesis in the grape berry starts after flowering, reaching the maximum concentration two to three weeks before *véraison*, after which it declines during maturation, not only in concentration, but also on a per berry basis.[13,16]

Recent studies suggest that IBMP found in the berry is synthesized by the berry



Berries harvested well before sugar maturity are widely reported to have higher levels of methoxypyrazines.

Figure 2: Opening the canopy at pea size (red arrow) affects the biosynthesis of IBMP in the berry, which in turn results in a reduced IBMP concentration in the wine (curve adopted from Roujon de Boudee, 2000).[14]

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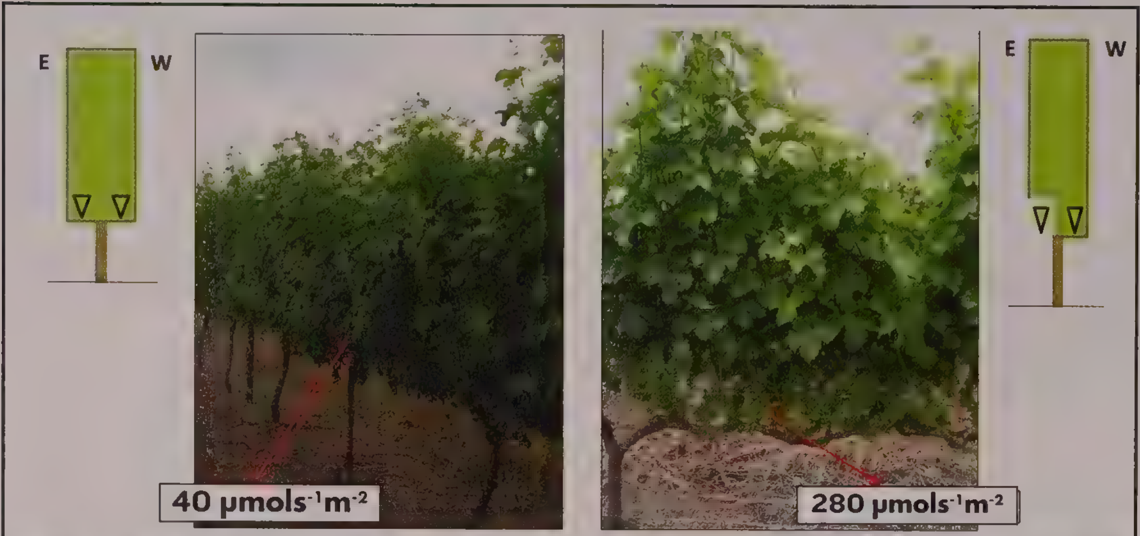

Figure 3: Leaf removal performed on Sauvignon Blanc vines at phenological stage berry pea size. Left: The shaded treatment (no leaf and lateral shoot removal) with a mean daily PAR (photosynthetically active radiation) of $40\ \mu mols^{-1}m^{-2}$. Right: The morning side exposed treatment with a mean daily PAR of $280\ \mu mols^{-1}m^{-2}$.

itself and not transported to the berry from either the leaves or shoots.[9] This is not in agreement with D. Roujou de Boubée who has reported that deuterated analogue of IBMP was translocated from the leaves to grape clusters.[14]

Abiotic factors such as light and temperature in the fruit zone and the vine water status, and various viticultural practices such as leaf removal, irrigation and vine training system can influence the concentration of IBMP in the berry and wine.[2,14,17] It has been shown that grapes and wines from cooler climatic regions contain higher concentrations of IBMP than grapes grown in warmer regions.

Recent studies have shown the importance of the timing and severity of leaf removal on the final concentration of IBMP in the mature grapes. J.J. Scheiner *et al.*, have shown that early leaf removal, performed 10 days after flowering, significantly reduced the IBMP concentration in Cabernet Franc grapes, while the same treatment, applied 40 and 60 days after flowering, had a less significant effect.[18]

Leaf removal performed after veraison had little or no effect on the IBMP concentration in grape berries. Bunch exposure pre-veraison is therefore crucial for reducing IBMP concentration in grape berries at harvest, affecting mainly the synthesis. However, light exposure after veraison does not influence IBMP degradation.[16,18,19]

To understand the effect of sunlight exposure to bunches on the IBMP concentration in grape berries during maturation and the resulting concentration in the wine, leaves and lateral shoots were removed on Sauvignon Blanc and Merlot vineyards in the Overberg and Stellenbosch regions (South Africa).

MATERIALS and METHODS

Sauvignon Blanc

The experiment was performed in a *Vitis vinifera* L. cv. Sauvignon Blanc (clone 316, grafted onto 101-14 rootstock) vineyard in the Overberg region of the western coastal area, South Africa (34°S; 19°E). The row orientation is northwest to southeast with

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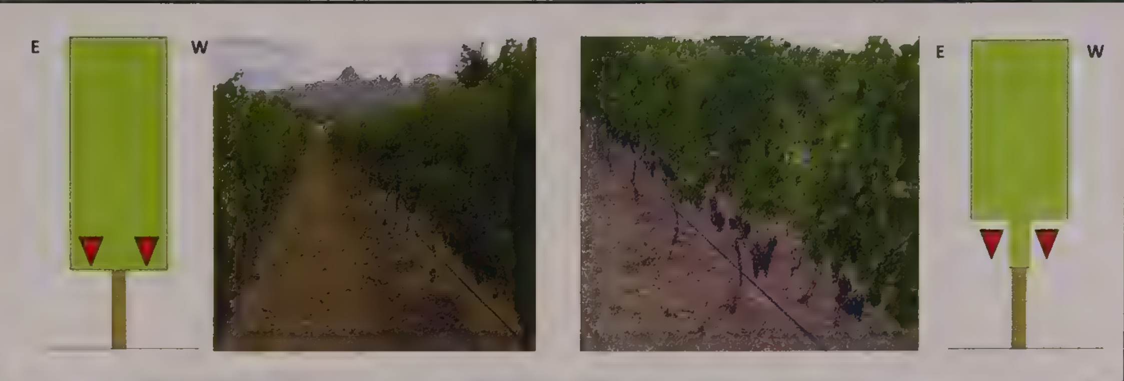

Figure 4: Leaf removal as performed on Merlot vines at the phenological stage of berry pea size. Left: The shaded treatment (no leaf and lateral shoot removal). Right: Both sides of fruit zone exposed. This extreme treatment has been done for experimental purposes only. The site, row orientation, and irrigation management made it possible to avoid berry sunburn or berry shriveling. Normally, opening the canopy on one side of the fruit zone is sufficient to achieve lower IBMP concentrations in wine.

2.5 m x 1.8 m vine spacing. The training system is VSP (vertical shoot positioning) with a bilateral cordon and six two-bud spurs per running meter of cordon.

The treatments were randomly located within a greater experimental layout to exclude the natural heterogeneity of the vineyard and consisted of eight panels (four vines per panel) each.

To study the impact/role of light and temperature, all leaves and lateral shoots were removed from the fruit zone on the morning side of the canopy to a height of 0.3 to 0.4 m above the cordon. The 100% morning side exposed bunches were compared with a control canopy (100% shaded bunches where no leaves or lateral shoots were removed). Leaf and lateral shoot removal was performed at the phenological stage of berry pea size on Dec. 19, 2011.

Stem water potential (Ψ_{SWP}) was used to determine vine water status.[7] The vines did not experience any water constraint during the growing and ripening period with a mean SWP of Ψ_{SWP} -450 kPa at *véraison*.

Micro-vinification of the two treatments was performed in triplicate in the Stellenbosch University experimental cellar with standardized methods. For the exposed treatment, only exposed bunches were harvested, whereas the complete canopy was harvested for the shaded treatment.

Sensory analyses were performed five months after bottling using descriptive analyses. The wines were tasted in triplicate, randomized per taster and presented in black glasses. Aromatic descriptors, generated by the tasting panel during training, were scored on an uncalibrated line scale with the aromatic detection thresholds ranging from "none" to "intense."

Merlot

The Merlot vineyard is in the Helderberg area, Stellenbosch (33°S; 18°E) in the Western Cape, South Africa. The Merlot vines (clone 348A grafted onto US8-7 rootstock) were planted in 2003. The row direction is east to west with vines spaced 2.4 m x 1.2 m. The trellis system is a VSP on a bilateral cordon with five two-bud spurs per running meter of cordon.

Pre-dawn leaf water potential (Ψ_{PD}) and stem water potential (Ψ_{SWP}) were used to determine vine water status and to manage irrigation, maintaining the vine water status at around -400 to -500 kPa for the Ψ_{PD} values.[7]

To study the effect of light and temperature on the berry IBMP concentration, leaves and lateral shoots were removed at the berry pea size phenological stage. All leaves and lateral shoots were removed on both sides of the canopy to the height of the first wire (100% exposed bunches). No leaves or lateral shoots were removed in the shaded treatment (100% shaded bunches) as a control. The grapes were harvested according to the sugar loading model at the fresh and mature fruit stages.[6,8]

RESULTS and PERSPECTIVES

Sauvignon Blanc

The Sauvignon Blanc vineyard is exposed to the positive effect of the sea breeze, coming from the Atlantic Ocean, on the bunch microclimate. Microclimatic data was collected in the fruit zone, whereas climatic data collected from above the canopy was considered as mesoclimatic data.

The sea breeze allowed for the canopy to be opened in the fruit zone, and second to partially separate the effect of light and temperature on berry composition due to the cooling effect of the sea breeze.[4] The coolest temperature in both treatments was measured at 6 AM. The mean hourly temperature over the growing and ripening season for both treatments did not exceed 30.5° C, where 30° C is the upper limit of the temperature threshold for optimal vine functioning.

Due to the vineyard location, it was possible to open the canopy without experiencing any sunburn due to the occurrence of the sea breeze (see Figure 5). An increase in wind speed was observed from 10 AM onward, reaching a maximum speed between noon and 6 PM. The increase in wind speed resulted in a decrease in the ambient (mesoclimatic) and bunch temperatures, mainly from 1 PM onward (Figure 5).

Wind direction measurements (data not shown) confirmed that the wind direction was predominantly from the south (Walker Bay) and from the west

Treatment	Total soluble solids (°Brix)	Titratable acidity (g/L)	pH	Berry fresh mass (g)	Sugar per berry (mg)
Morning side exposed	24.4	6.53	3.39	1.95	475.81
Shaded	23.4	7.38	3.31	1.97	460.43

Table I: Basic Sauvignon Blanc grape berry maturity parameters for shaded and morning side exposed treatments at harvest (3/13/2012).

Merlot	Sampling dates	Harvest stages	°Brix	Titrable acidity	pH	Berry fresh mass of one berry (g)	Sugar per berry (mg)
Expo-both sides	2012/02/10	fresh fruit	23.4	5.74	3.46	1.58	370.83
	2012/02/20	mature fruit	25.2	4.94	3.51	1.61	406.23
100% Shaded	2012/02/10	fresh fruit	23.1	6.22	3.40	1.61	373.03
	2012/02/20	mature fruit	25.0	4.83	3.44	1.61	402.88

Table II: Basic grape berry maturity parameters for shaded and both sides exposed treatment for grapes harvested at fresh fruit stage (2/10/2012) and mature fruit stage (2/20/2012).

(False Bay) during the noted hours. The main effect of this sea breeze was to cool down the berry and canopy temperature without directly affecting stomatal conductance.

The treatments did not significantly affect the berry fresh mass and main grape berry maturity parameters at harvest (see Table I).

Fifty percent flowering occurred Nov. 20, 2011 (from here on referred to as the date of flowering), *véraison* (50% of berries softened) was Feb. 2, 2012, and the grapes were harvested on March 13, 2012. The concentration of IBMP was analyzed in whole grape berries, twice before *véraison* (53 and 59 days after flowering), around *véraison* (73 days after flowering), during ripening (94 days after flowering) and at harvest (113 days after flowering).

For the morning-exposed treatments, only exposed berries were collected for MPs analyses and shaded treatment berries were sampled randomly within the entire canopy.

Leaf and lateral shoot removal from the fruit zone on the morning side resulted in lower IBMP concentrations during ripening (Figure 6). Significantly higher concentrations of IBMP were observed in the shaded treatment compared to the morning-exposed treatment for samples at 51, 59 and 73 days after flowering. At 94 days after the date of flowering and at harvest, no significant differences were noticed between the treatments.

A rapid decrease in the IBMP concentration in grape berries for the first three sampling dates of the morning-exposed treatment was observed, that could be a result of a lower IBMP synthesis in the grape berry which is most probably a consequence of early leaf and lateral shoot removal (berry pea size phenological stage).[17]

From 94 days after the date of flowering to 113 days after flowering (harvest), little change was noticed in the IBMP concentration of the morning-exposed treatment. Whereas the concentration of IBMP in the shaded treatment decreased slowly up to harvest for the same time period.

The final IBMP concentration in the grape berry at harvest can differ significantly between harvest seasons, being dependent on both leaf removal (direct or indirect effect of light in the fruit zone) and the climate (effect of temperatures: average, maximum and minimum including temperature differences between day and night).[10]

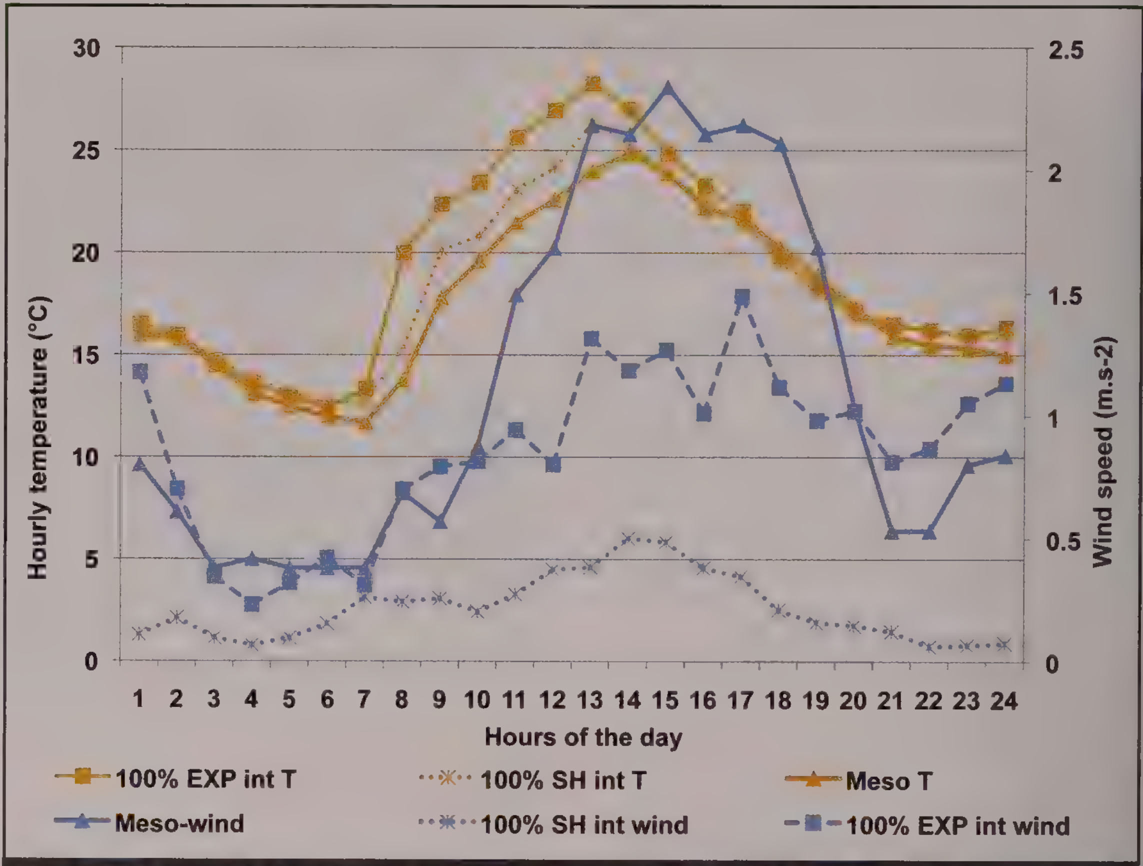

Figure 5: An example of sea breeze and temperature evolution in 24 hours. Figure 5 represents wind speed at the mesoclimatic level (Meso-wind), in the fruit zone in the morning side-exposed treatment (100% EXP interior wind), and in the fruit zone in the shaded treatment (100% SH interior wind). Temperature evolution has been recorded at the mesoclimatic level (meso-T), in the fruit zone for the morning side exposed treatment (100% EXP interior-T) and shaded treatment (100% SH interior-T). Climatic data collected above the canopy were considered as the mesoclimatic data, whereas microclimatic data were collected at the fruit zone. The mesoclimate could be defined as the climate at the vineyard level.

The interaction between light and temperature in the fruit zone is complex and the goal of this study was to clearly show the direct effect of light intensity on fruit IBMP concentration and the very complex and indirect relationship between fruit and wine composition and wine sensory description.

The direct effect of the vintage that was observed, compared to the effect of temperature, was due to the effect of the heat waves and time and duration of the heat waves during fruit development and ripening. This allowed demonstration of the overlapping effect of temperature irrespective of the light intensity at the fruit level, even in a site (*terroir* unit) where the sea breeze cooled down the fruit temperature during the hottest hours of a day.

IBMP concentrations in produced wines were lower than what was found in the grapes as was expected due to the decrease in IBMP after juice clarification as previously observed.[14] No significant difference in the IBMP concentration in grape berries between both treatments was observed at harvest, whereas the IBMP concentrations in the wines were higher in the shaded treatment when compared to the morning side exposed treatment (Figure 7).

Slightly higher, but not significantly, IBMP concentrations in grape berries from the shaded treatment could explain higher IBMP concentrations in the wines. Due to the natural heterogeneity within the vineyard that can counteract the effect of the treatments, the larger winemaking sample (at least 50 kg of grapes) may be more representative in comparison to 100 berries used for grape berry analyses. The IBMP concentration in wine from the shaded treatment was above the detection threshold (2 ng/L), corresponding with the attributes detected during sensory wine evaluation.

Figure 8 shows the separation in wine sensory perception between the shaded and the morning side exposed treatment, with green attributes mainly linked to the shaded treatment (no leaf removal) and tropical fruity attributes linked to the exposed treatment (morning side exposed bunches).

Using principal component analyses (PCA), descriptors such as green pepper, grassy and overall greenness were grouped with IBMP. Tropical descriptors (grapefruit, passion fruit and guava) were mainly grouped with varietal thiols such as 3-sulfanyhexan-1-ol (3SH), 3-sulfanyhexyl acetate (3SHA) and some

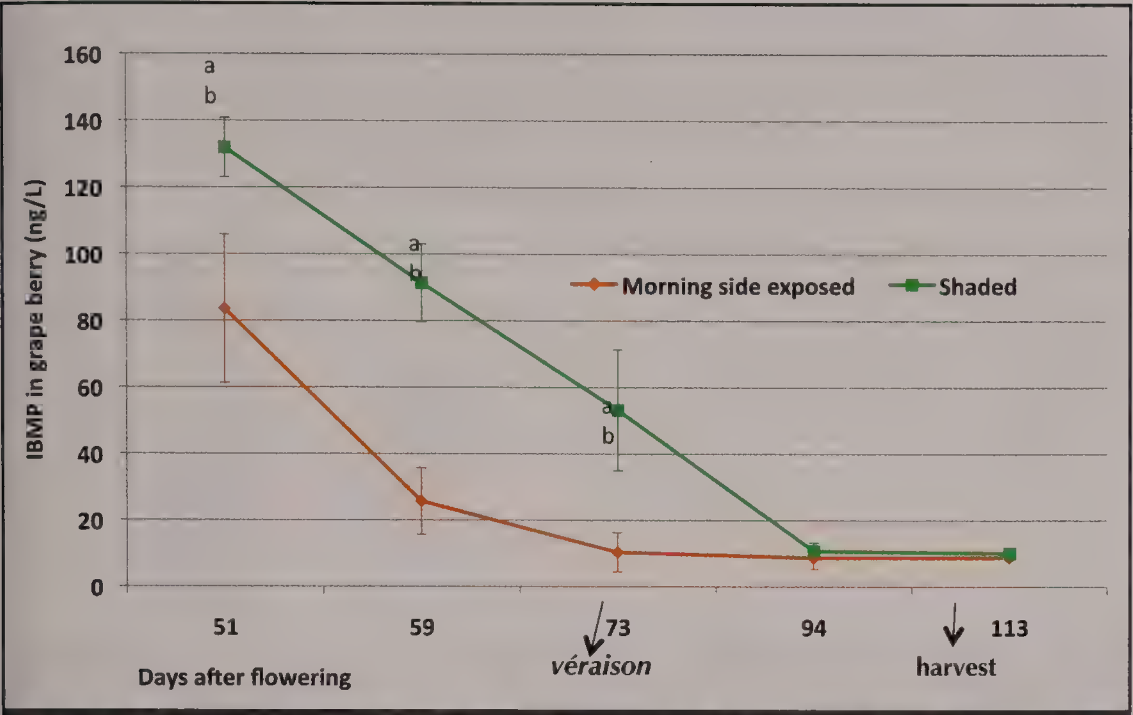

Figure 6: The concentration of IBMP (ng/L) in Sauvignon Blanc grape berries from 53 days after flowering (Jan. 12, 2012) to harvest, 113 days after flowering (March 13, 2012) for the shaded and the morning side exposed treatments.

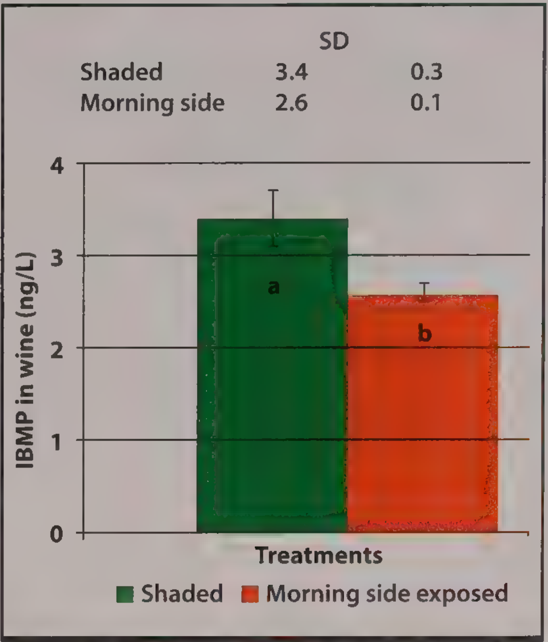

Figure 7: The concentration of IBMP (ng/L) in Sauvignon Blanc wines from shaded and morning side exposed treatments.

esters. By removing leaves and lateral shoots, a more complex (fruitier) style of Sauvignon Blanc was produced in comparison to the one-dimensional (greener) wine style produced from the shaded treatment.

Merlot

The treatments did not affect the berry fresh mass and classical ripening parameters irrespective of the harvest dates at fresh and mature fruit stages (Table II). The increase in Brix between fresh fruit and mature fruit stages can be attributed to an increase in sugar per berry.

The IBMP concentration in Merlot grape berries decreased during maturation. A higher concentration of IBMP in grape berries was observed in the shaded treatment (no leaves or lateral shoots removed), compared to the both sides exposed treatment, where leaves and laterals were removed at the berry pea size phenological stage (Figure 9).

At the last three sampling dates for both treatments, although no significant decrease in the IBMP concentration was observed, a lower IBMP concentration was noted for the both sides exposed treatment compared to the shaded treatment.

IBMP concentrations in the wines were higher for the shaded treatment than the both sides exposed treatment, that was also observed in the grape berries (Figure 9). No noticeable difference was seen in the IBMP concentration between wines for the two harvest stages of fresh and mature fruit (Figure 10).

The IBMP concentration in grapes remained relatively stable after reaching a certain concentration.[16] The both sides exposed treatment resulted in wines with an IBMP concentration of less than the detection threshold of 15 ng/L in red wines, whereas the IBMP concentration in the wines of the shaded treatment was 18.1 and 18.3 ng/L respectively for grapes harvested at the fresh and mature fruit stages (Figure 10).[13]

Conclusions

Increased light in the fruit zone has an effect on berry composition, generally

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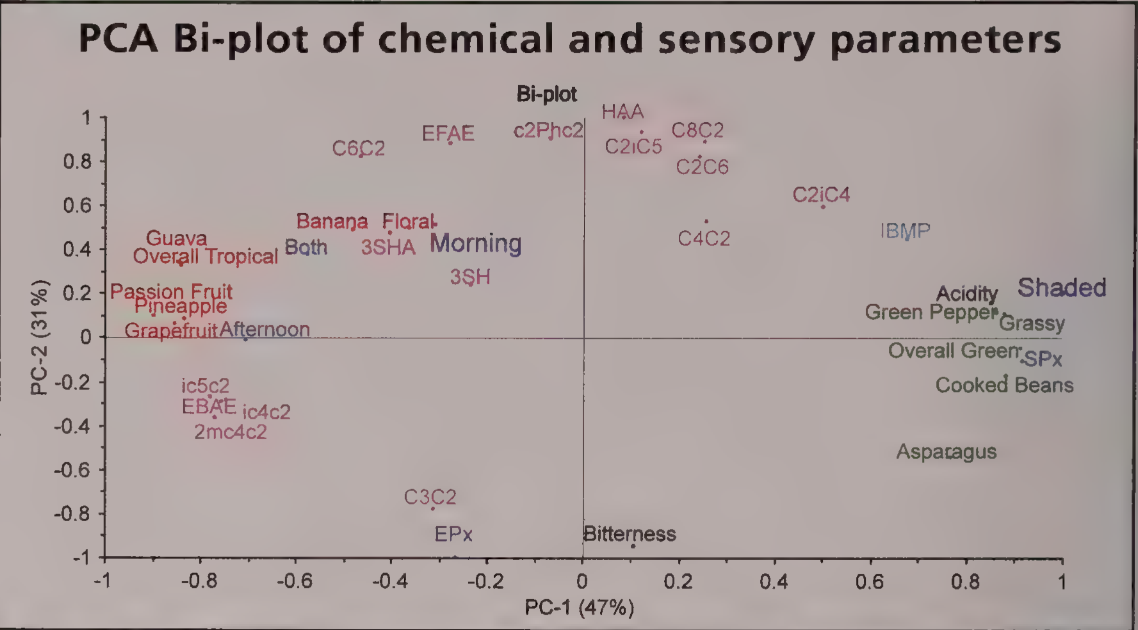

Figure 8: A PCA plot indicating the distribution of the treatments according to the sensory analyses results and chemical analyses where the morning side exposed treatment is indicated as "Morning" and the shaded treatments as "Shaded." PC-I represents 47% variability when PC-2 represents 31% of variability. The figure shows a clear difference between wines from shaded and exposed treatments: a more one-dimension green-style wine, associated with IBMP, from shaded treatment, and a more complex wine from exposed-bunches treatment, associated with thiols.

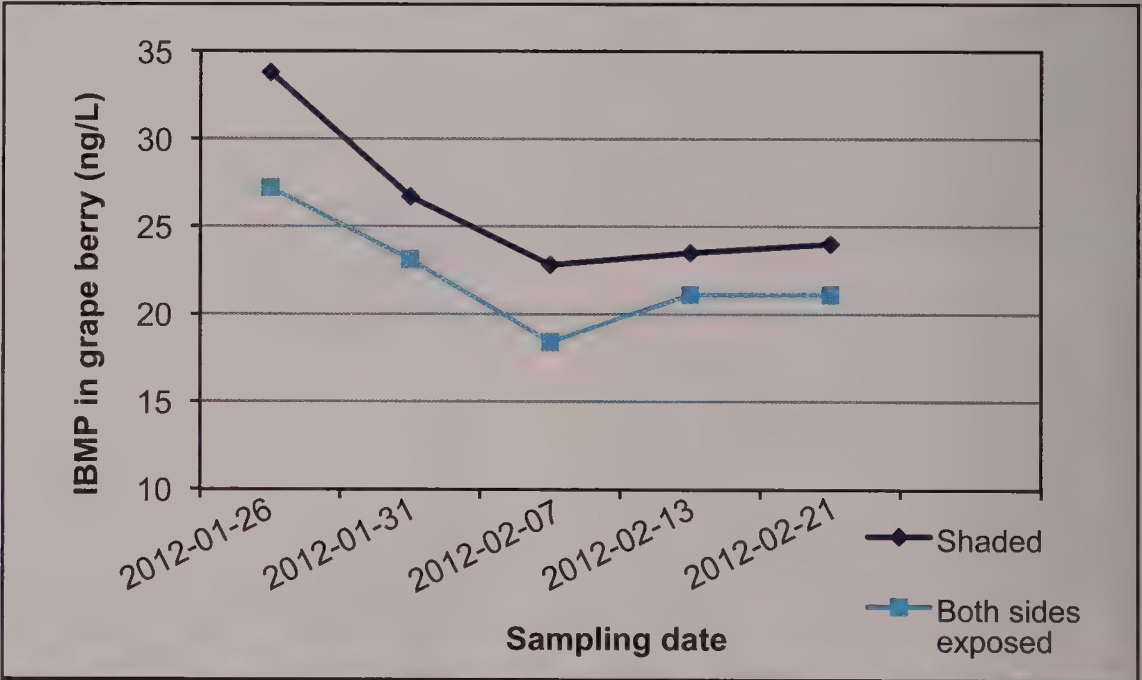

Figure 9: The concentration of IBMP (ng/L) in Merlot grape berries from Jan. 26, 2012, to harvest (Feb. 21, 2012) for the shaded and both sides exposed treatments.

improving the resulting wine quality by decreasing IBMP concentration and increasing anthocyanins and polyphenols in the wine,[15] and enhancing the fruitiness of the wine.

IBMP synthesis and degradation patterns are complex and can be influenced by many environmental conditions and canopy management practices. Early leaf removal (at the berry pea size phenological stage) is crucial to reduce the IBMP concentration in the grapes.[9]

Temperature is as important for the IBMP concentrations in grapes and can counteract the effect of light intensity in the fruit zone.[10] Furthermore, high IBMP concentrations in Sauvignon Blanc wines may mask the fruitiness of the wine and may be considered a negative aroma, although mainly in red wines like Merlot and Cabernet Sauvignon.

To reduce the pyrazine level in the berries and consequently in the wine, early (berry pea size) canopy manipulation can be applied in the fruit zone by removing all leaves and lateral shoots at a height of 0.3 to 0.4 m above the cordon in a VSP training system.

In Sauvignon Blanc grape berries, no significant difference in the IBMP concentration at harvest was observed, whereas higher IBMP concentrations from the shaded treatment in resulting wines were found. IBMP degradation patterns can differ between vintages and can sometimes overcome the effect of bunch sun exposure, in combination with the average temperature of the vintage and number, time of occurrence and length of heat waves experienced in the South African grapegrowing region.[2,10]

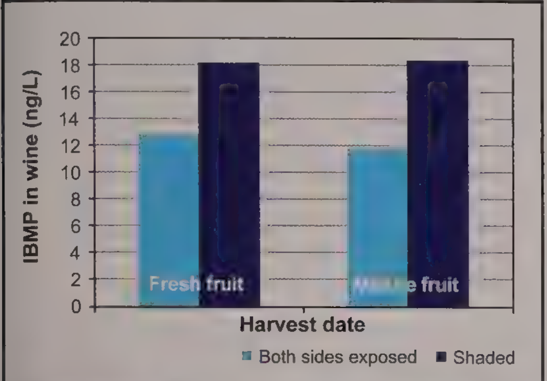

Figure 10: The concentration of IBMP (ng/L) in Merlot wines for the both sides exposed and shaded treatments, harvested at fresh and mature fruit stages. No decrease in IBMP concentration was observed during ripening from fresh to mature fruit stages, for both treatments. A noticeable difference in wine IBMP concentration was observed between the treatments: ~12ng/L for wine from exposed bunches and ~18 ng/L for wine from shaded bunches.

- Removing all leaves and lateral shoots in the fruit zone on one side of the canopy is an effective tool to reduce IBMP concentration in a wine. The choice of the canopy side to remove leaves has to be chosen properly according to row orientation and site location to prevent possible sunburn in warm climatic conditions. The timing of leaf removal is crucial to reduce IBMP concentrations in grapes. Leaf removal performed before *véraison* (at berry pea size) results in a significant decrease of the IBMP concentrations in grapes, whereas treatments applied after *véraison* had little or no effect on IBMP concentration in grapes.[10,14,16]
- By removing only laterals from the fruit zone and retaining basal adult leaves of the primary shoot intact, sufficient light will be allowed into the fruit zone. Selective opening of the fruit zone would however, be more time consuming than removing both laterals and basal leaves simultaneously.
- In a warm/hot climate, similar results can be obtained by indirectly increasing light in the fruit zone using a modified VSP trellis such as the Smart Dyson, the sprawling VSP (allowing light to penetrate the canopy and to reach bunches through the top of the canopy/diffuse light penetration) or implementing a new Lys training system.[5]

Early leaf removal before *véraison* seems to be an efficient tool to reduce the concentrations of IBMP in grape berries. As proposed by other authors, the IBMP concentration in the grape berry is not only light- but also temperature-dependent, which could, according to the site and vintage details, counteract the effect of the light intensity. Further research on this topic is needed, mainly to understand the complex relationships between fruit and wine composition, and ultimately wine sensory description. PWV

Acknowledgements

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Functions and benefits of OAK BARRELS for fermentation/*élevage*

BY Nicolas Tiquet-Lavandier, Marie Mirabel, Taransaud Tonnellerie, Cognac, France

What is an oak barrel for? The French word *"élevage,"* which means bringing up, can give us a clue. It is to achieve the potential of a wine through maturation and to enrich it with new characteristics that come from oak. This involves a long process that is respectful of the nature of the wine.

Barrels can be used for alcoholic and malolactic fermentation prior to *élevage*, but it is quite unusual to use barrels for fermentation only and not for *élevage* afterwards. Indeed, most often barrels are used mainly for *élevage*. What occurs during *élevage*?

What are the functions of barrels and what benefits can we expect from them?

Barrels serve to:

- Enrich the wine with new compounds,
- Play a role as an interface between the wine and the environment, and
- Allow specific physical reactions to occur.

First function — Enriching the wine with new compounds

Which compounds are released into a wine and how do they improve a wine? To answer these questions we need to look more closely at the raw oak and its chemical composition.

a. Raw oak composition — Different polymers are clearly identified in oak

Figure II shows the chemical structure of these different compounds.

Two sugar polymers are present. Cellulose represents 40% of oak's chemical composition and contributes to its strength, and hemicellulose represents 25%. Both of these sugar polymers provide support. There is also a phenol polymer called lignin, which represents 25% of the oak and helps to add structure to the oak. These polymers will not be released in the wine, but they will be transformed during the barrel-making process.

Some extractable components are also present, that are releasable. About 10% of the oak components are phenolic compounds (tannins, phenols, coumarins — bitter phenols, etc.). Between 1% to 5% are represented by other components such as minerals, terpenes and aromatic compounds.

The geographical origins of the oak, the forest where it comes from, and the type of grain will influence the chemical composition of the wood.

b. Compounds released from oak into the wine

There are two main categories:

Aromatic compounds such as lactones, eugenol and vanillin that will contribute to the flavor of the wine. Wood polyphenols and ellagitannins will contribute to the structure and taste of the wine.

First, in terms of flavor, different aromas are identified:

- Furfural (almonds and smoke),
- Methyloctalactone (coconut),
- Eugenol, isoeugenol (cloves and spices),
- Vanillin (vanilla),
- Maltol, cyclotene (caramel and toffee),
- Guaiacol (smoky notes).

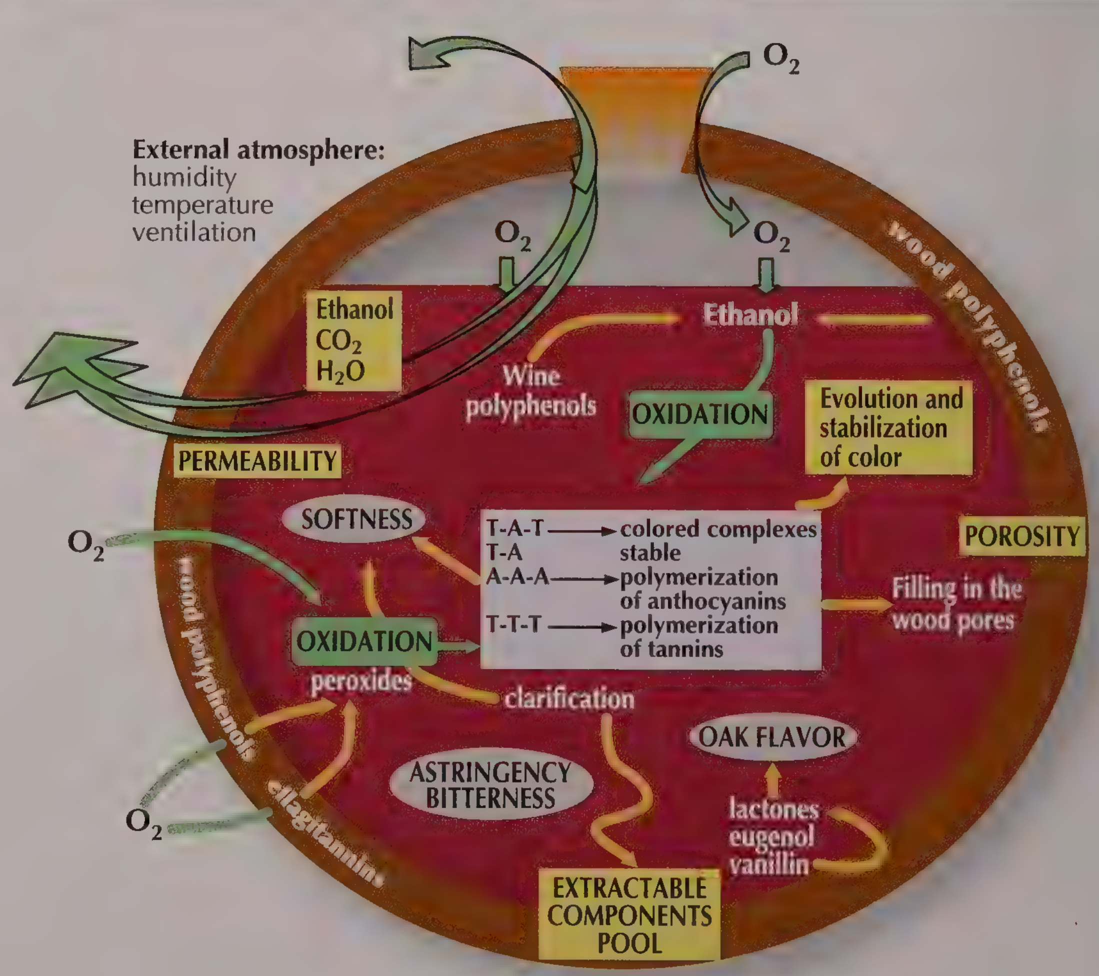
The bouquet of a wine aged in barrels is therefore richer and more complex than that of unoaked wine.

Second, some compounds influence barrel taste and mouthfeel. Tannins from wine grapes represent between 2 and 4 g/L. But oak tannins extracted by ageing in a barrel can be up to 0.2 g/L. These oak tannins have strong antioxidant properties. They make the wine more tannic (enriching its structure) and they also make it tastier.

At the same time, other components add some sweetness to the wine.

The cooperage processes, such as the seasoning of staves and the different barrel-making steps including toasting, will change the oak composition and, in particular, the nature and concentrations of the above-mentioned compounds.

Figure I: Reactions and exchanges with the barrel as membrane.



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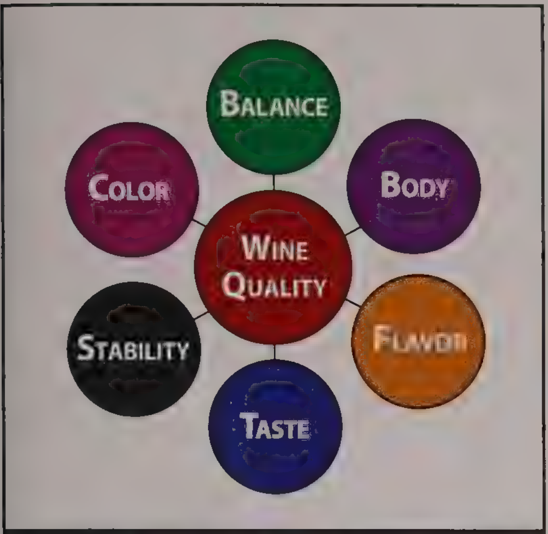

Figure III: Benefits of *élevage*.

Second function — The barrel is a membrane between wine and the cellar environment

When we discuss the environment of a barrel, we refer to the cellar's humidity, average temperature, and ventilation.

a. Oxygen

Oxygen in the atmosphere will interact with wine in a barrel. The permeability and porosity of the barrel allow for several interactions:

- Oxygen can enter the barrel between the bung and the staves and then dissolve in the wine.
- Oxygen can travel through the wood of the barrel.
- Oxygen can enter between the staves into the wine.

Different steps in the winemaking process also allow oxygenation to occur. The following statistics are what we found in different sources in the technical literature.

- During each racking, 6 mg/L of oxygen dissolves into the wine,
- Each topping up of a barrel adds around 4 mg/L to the wine, and
- Maturation itself — the transfer of oxygen when the wine remains in the cask — can represent 35 mg/L.

In summary, a large amount of oxygen is brought to a wine during *élevage* in barrels, through the barrels themselves and during different steps of *élevage* (racking and topping up).

b. Reactions allowed by this interaction with oxygen

Barrels in the cellar are interacting with the environment. Oxygen crosses the limits of the barrel through different pathways, and is diluted into the wine. Oxygen allows ethanal to be produced in the wine. Both oak and wine polyphenols can interact with ethanal and generate different combinations: between tannins, between anthocyanins, and between tannins and anthocyanins together.

The consequences of these chemical reactions are the following:

- The color of the wine is stabilized and its intensity increases,
- There is a decrease in the bitterness and astringency of the wine, and
- Tannins are transformed to give the wine its softness.

c. Other exchanges through the membrane

One must consider porosity to gas and permeability to liquid and gas. If it works one way for oxygen (from outside to inside the barrel), it can work the other way for components such as ethanol, water, and gas as carbon dioxide. Although there is an apparent loss, the wine becomes more concentrated — this phenomenon is called the "angels' share."

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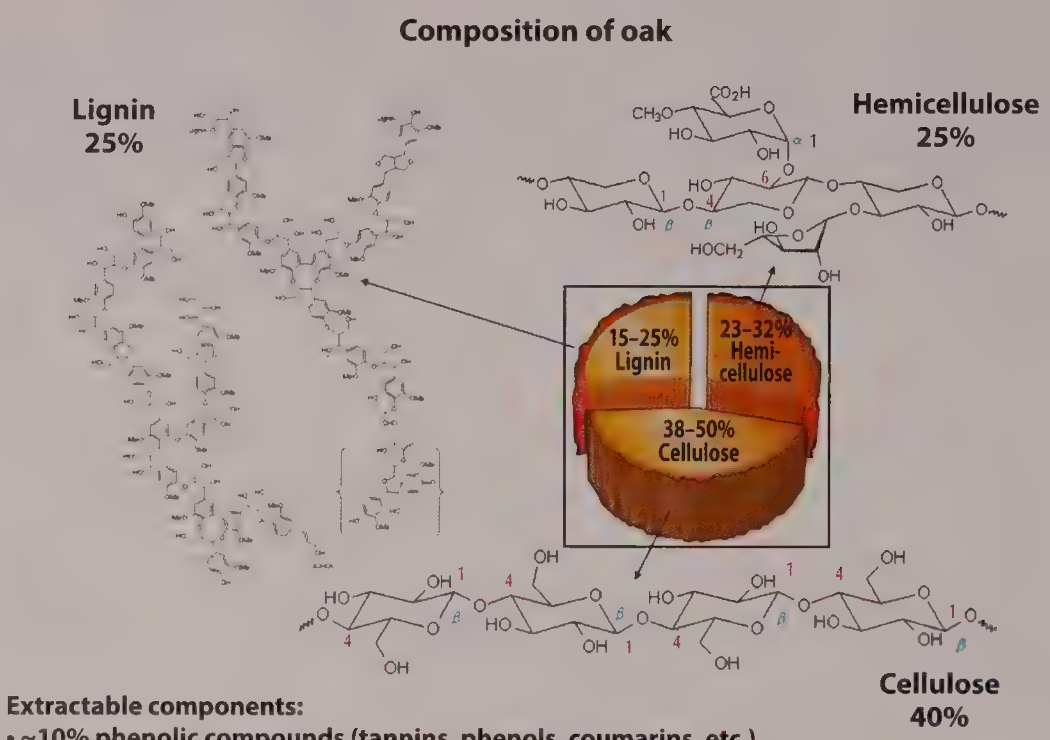

Extractable components:

- **~10% phenolic compounds (tannins, phenols, coumarins, etc.)**
- **1-5% other components: minerals, terpenes, aromatic compounds, etc.**

This composition depends on quality factors and selection of the trees, and will be modified by the different cooperage processes.

Figure II: Adding oak compounds to wine.

Third function — Physical properties of oak allow certain reactions to occur

Barrels are good in that they stabilize and protect the wine in them. Indeed, oak vessels limit variations in temperature as their temperature conductivity is more than 100 times less than stainless steel.

Because barrels are small vessels, they allow certain colloidal reactions to occur. Among these reactions, the precipitation of unstable compounds allows the clarification of the wine. It is also possible to do *élevage* (the ageing on lees known as reductive ageing), which gives the wine some body and sweetness.

Conclusion

The main benefits of ageing (*élevage* of) wine in a barrel are:

- Improves the wine's stability,
- Deepens the color of the wine,
- Gives the wine more balance,
- Contributes to the taste of the wine,
- Helps the body become fatter, and
- Adds to the flavor of the wine.

All these different parameters determine a great part of wine quality, making barrels a crucial tool for winemaking and contributing to wine style. PWV

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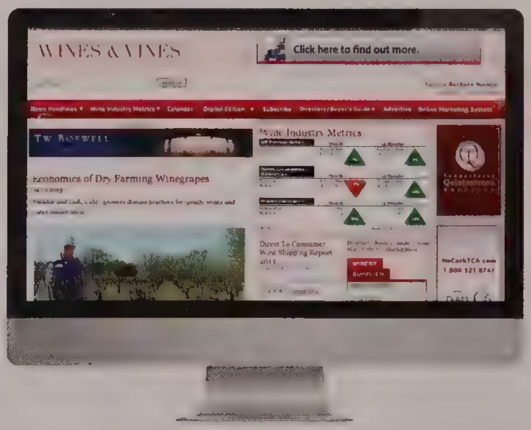
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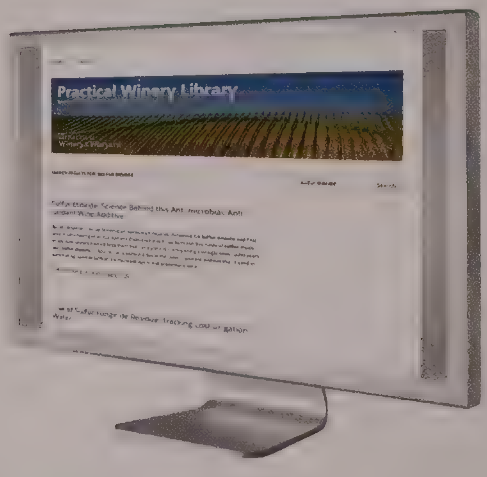
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NITROGEN
Friend or foe of RED WINE QUALITY?

BY **Markus Keller**, Washington State University, Prosser, Wash.

Yield is determined by the amount of carbohydrates (sugar) partitioned to the fruit rather than to other organs of grapevines. The classical view of the relationship between grape yield and quality is that of a linear decrease in quality as yield increases. Moreover, many grapegrowers believe that the more they stress their vines, the better the resulting fruit quality will be.

However, reality is not that simple. A more scientific view is that of an optimum curve with initially increasing quality, followed by a plateau, and then a reduction in quality when yield is further increased. Under changing external conditions, this curve can be shifted upward or downward.

Rather than setting a specific, inflexible target yield, it should be the goal of every economically thinking vineyard manager to achieve the highest yield possible without compromising quality.

Roles of nitrogen

Nitrogen is only one of a whole range of tools available to vignerons to manage the balance between yield and quality. Other tools include vineyard design (row orientation, planting distance, trellis design, training system), pruning (node number and position), canopy management and soil and water management.

However, of all mineral nutrients, nitrogen is the most potent in terms of influencing vine growth, morphology and tissue composition. This is primarily because nitrogen is a chemical component of many critically important plant constituents. Nucleic acids contain it, and they in turn make up the genetic information contained in the vine's DNA.

Nitrogen is also in amino acids, which when linked together make up the proteins and enzymes that drive all biochemical reactions. It is an integral part of chlorophyll, responsible for intercepting and capturing sunlight, of hormones used for communication between different plant organs, and of certain secondary metabolites, some of which also contribute to wine flavor.

This text edited from first publication: Sept./Oct. 2005.

Nitrogen is present in the soil solution in the form of nitrate (NO_3^-) and ammonium (NH_4^+) ions. Nitrate is the vine roots' nitrogen uptake form of choice. It is reduced to ammonium in the roots and metabolized (assimilated) into the amino acids glutamine and glutamic acid.

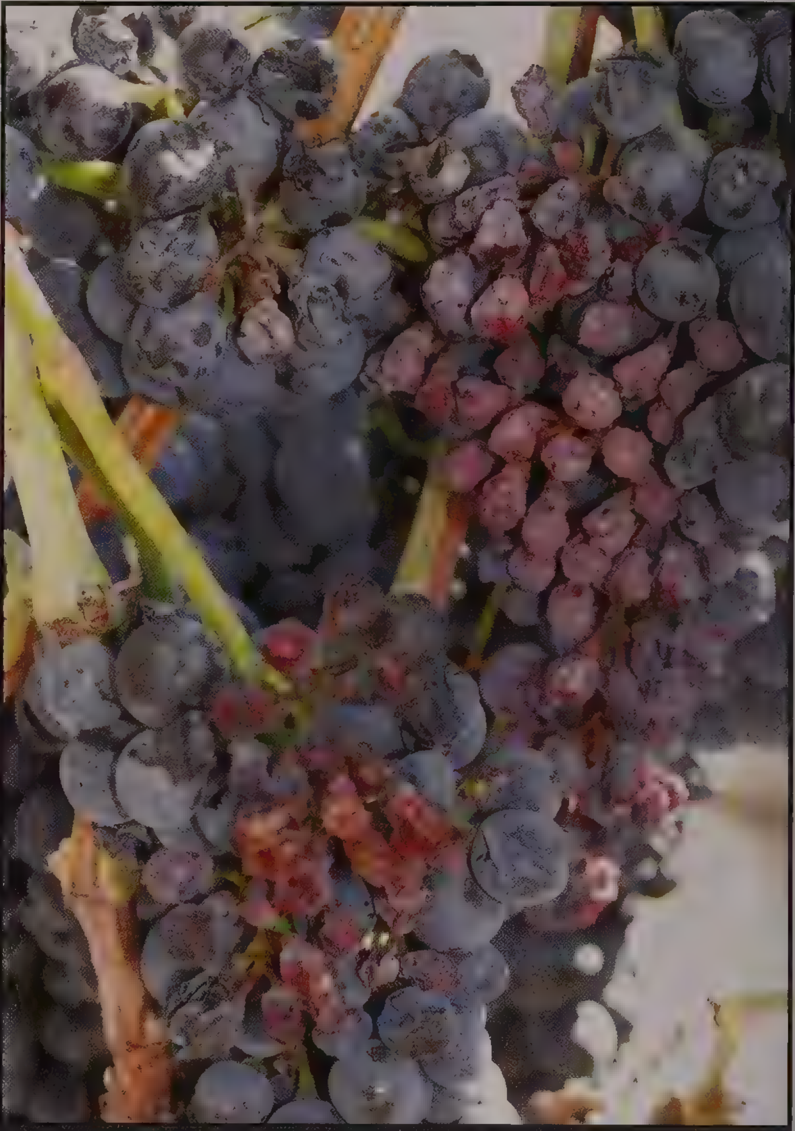
Glutamine is the main nitrogen transport form in the transpiration stream (xylem), but with increasing nitrogen supply in the root zone, nitrate is increasingly transported to the shoots in addition to glutamine.[2,5] This is because nitrogen uptake and assimilation in the roots are expensive in terms of carbohydrate requirements (both to fuel energy generation and as backbones for amino acids). Therefore, if nitrogen supply exceeds demand, the excess nitrate must then be assimilated in the leaves and fruit, which for them constitutes a conflict of interests.

Nitrogen (in chlorophyll and enzymes) stimulates photosynthesis in the leaves. Photosynthesis is the process by which the energy from sunlight is transformed into biochemical energy (ATP) and used to fix carbon dioxide (CO_2) and water (H_2O) to produce sugar (glucose). This glucose is stored as starch or made into sucrose, which is then exported in the phloem to various plant parts.

Increasing nitrogen supply enhances photosynthesis, which means that more sugar is available for growth and fruit ripening. However, when excess nitrate arrives in the leaves, it competes for carbohydrates and may result in a shift of the vine's priorities from fruit ripening to shoot growth.[9,10]

Nitrogen and vigor

Excessive vegetative growth (vigor) can lead to shaded canopies and, along with



PHOTOS M KELLER

Sunburn on Syrah berries; a common problem with overexposed grapes and excess nitrogen supply.

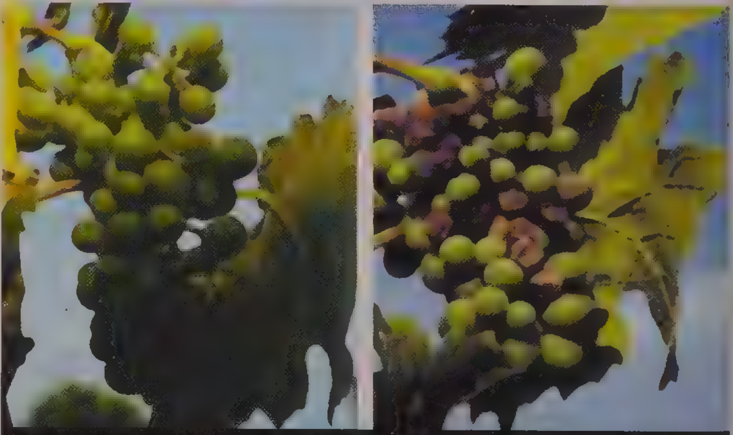


Figure I: Grapes grown on high-nitrogen soils are much more susceptible to sunburn (right), than grapes grown with low nitrogen availability (left).

the potential sugar shortage, reduced fruit quality. It is important, therefore, to develop a sufficiently large (roughly 10 to 15 square centimeters of leaf area per gram of fruit), sun-exposed canopy as early in the season as possible and then to stop shoot growth.

It's not only total grape color that is affected by nitrogen and weather factors, but also distribution of individual anthocyanins (red pigments).

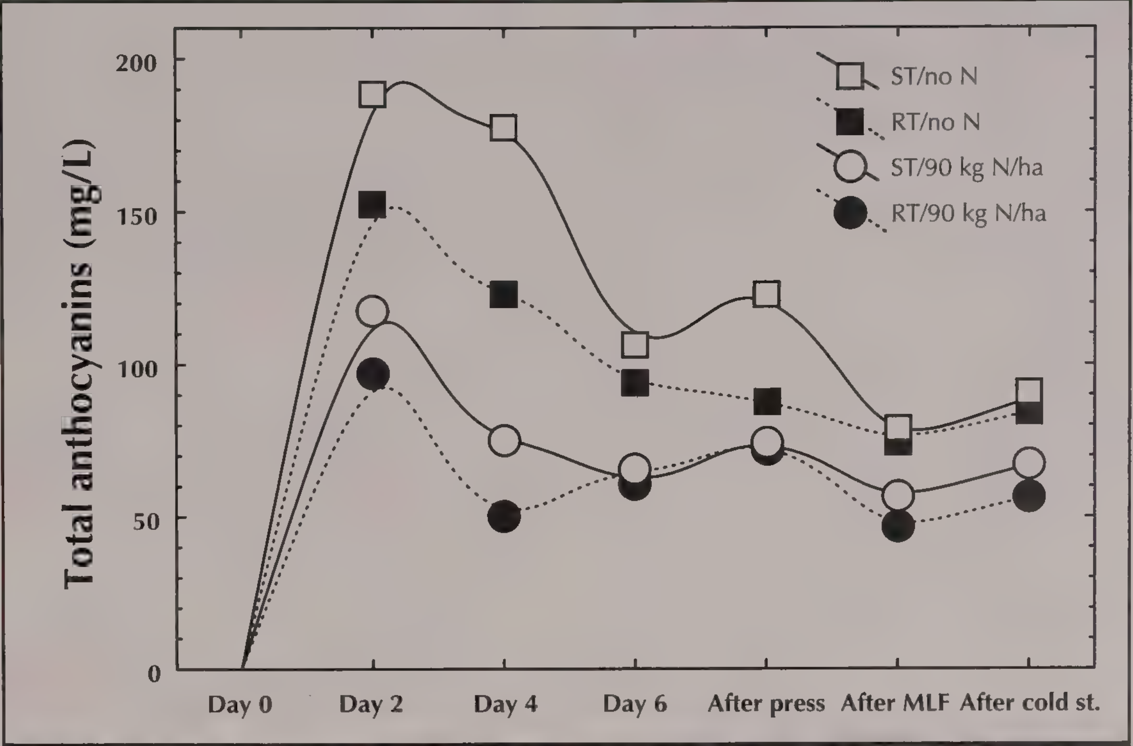


Figure II: The adverse effect on wine quality of excessive nitrogen supply during bloom cannot be alleviated by shoot topping (ST = single topping; RT = repeated topping) during the season. The development of wine color (anthocyanins) is shown from inoculation to cold stabilization (field-grown Pinot Noir, modified from Keller et al, 1999).

Sugar is not the only grape component that is adversely affected by shade.[9,10] Shaded grapes normally have less tartaric and more malic acid, which may result in an increase or decrease of pH.

Potassium content is often higher with follow-on effects on juice pH, while phenolic compounds, such as tannins and anthocyanins (red pigments), are reduced along with flavor compounds. This obviously affects the sensory properties of wine produced from these grapes; such wine often tastes thin and herbaceous.

To make matters worse, many fungal pathogens thrive in a shaded and humid microclimate, leading to an increase in disease incidence. This shade problem cannot be overcome by leaf removal in the cluster zone in an attempt to improve fruit exposure to sunlight, because the high nitrogen and low phenol content in the berries makes them susceptible to sunburn (Figure I).[9]

Another common Band-Aid action is to trim (often several times during the season) excess shoot tips, but again, this only makes matters worse, as shown in Figure II.[7]

It is ecologically and economically unwise to stimulate vigor by applying large amounts of nitrogen early in the season and then remove the surplus growth by summer pruning. Hedging wastes the vine's resources by stimulating lateral shoot growth and eliminating young, photosynthetically active leaves while leaving old, inefficient leaves behind.

How much is too much?

But how much nitrogen is good for the vine? The short answer is—it depends. In a warm, dry, sunny growing season, the optimum nitrogen supply may be higher than under cool, humid and cloudy conditions. See Figure III: the relationship between grapevine yield and fruit quality can be influenced by nitrogen supply and light conditions.[2,4]

Nitrogen deficiency during bloom results in poor fruit set and reduces a vine's yield potential. Despite low yield, however, fruit sugar concentration is below average, because there is insufficient nitrogen available for efficient photosynthesis. On the other hand, when clouds prevent sunlight from reaching the leaves, fruit quality is low regardless

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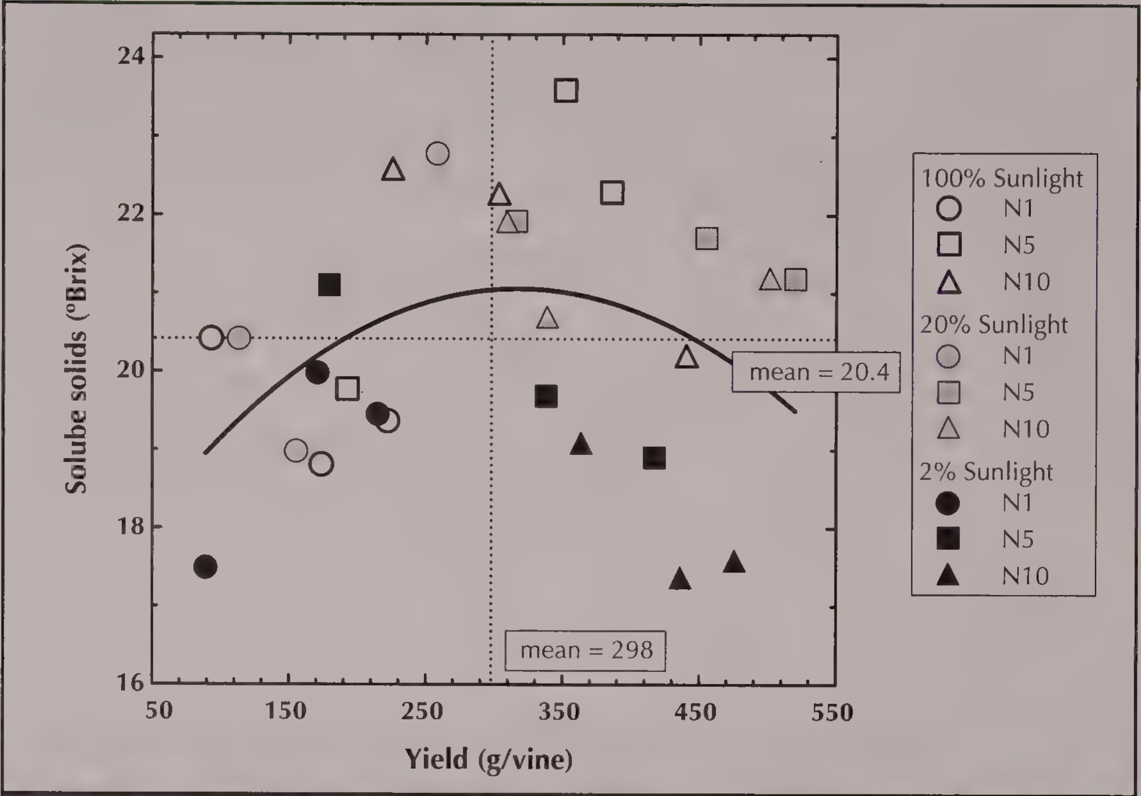

Figure III: Nitrogen supply and light conditions can influence the relationship between grapevine yield and fruit quality. Nitrogen was applied during bloom (N1 = 0.34 g N/vine; N5 = 1.7 g; N10 = 3.4 g), and light conditions were varied during veraison (pot-grown Cabernet Sauvignon, modified from Keller et al. 1998).

of nitrogen availability, because of lack of light for photosynthesis.

Yield and quality are maximized with sufficient nitrogen during the critical bloom period and a clear sky during veraison. Excess nitrogen can lead to poor fruit quality as discussed above. It can, moreover, increase the vine's susceptibility to low-light stress later in the season (Figure IV).

Nitrogen impacts color

Pigmentation of red grapes during *véraison* is maximized at moderately high temperatures and low to intermediate bloom-time nitrogen availability; it is minimized when heavy nitrogen supply is followed by overcast conditions.

Not only total grape color is affected by nitrogen and weather factors, but also the distribution of individual anthocyanins. Conditions favoring color accumulation (such as low nitrogen, high light and moderate temperature) may also lead to the most balanced distribution of pigments.

Because the formation of malvidinglucoside appears to be more tolerant of unfavorable environmental conditions than other anthocyanins, it becomes dominant in grapes grown in poor light or excessive heat, particularly in combination with excess nitrogen.[4,11]

Vine nitrogen status, therefore, has a direct influence on production of individual pigments in grape skins in addition to the indirect effect brought about by modifications of vigor and fruit set.[4,7] This has significant implications for winemaking, because the anthocyanin profile in grape skins determines the color potential of a wine.

The type and quantities of the different pigments influence both hue and color stability of a red wine. We can expect wine made from grapes that ripened in full sunlight on vines with relatively low nitrogen status to be deeply colored showing a well-balanced, crimson to purple hue. On the other hand, a decrease in total pigment content and a color shift toward red might be expected in wine made from grapes that experienced excessive nitrogen and poor light conditions.

Nevertheless, soil nitrogen availability should not be deficient during bloom. This period is critical because of rapid shoot and root growth, which can compete with developing flower clusters for available carbohydrates.[6] A second peak in root development appears to be the post-harvest period, especially in warmer climates.[1]

When to apply nitrogen?

Both bloom and post-harvest are therefore suitable periods for nitrogen augmentation, whether from mineralization of organic matter or from fertilizer application. Which one of the two periods is preferable depends on vine nitrogen status, grapegrowing region, weather conditions, and labor availability.

For example, post-harvest nitrogen application is an option only where leaves remain active on the vine long enough after harvest to enable nitrogen uptake. Regardless of the timing of nitrogen supply, nutrient availability is invariably linked to water supply, because nutrients can only enter the roots if they are dissolved in the soil water. Thus, water management, whether it involves rainfall, irrigation or cover crop management, is an important aspect of nitrogen management.[8]

Indeed water supply can sometimes be more important than nitrogen supply, especially in warm, dry climates. A comparison of irrigation strategies in conjunction with nitrogen application shows once again that the relationship between yield and quality is not a simple one.[12]

It seems that berry color is linked more closely to berry size than to actual yield, and berry size can be manipulated by irrigation. While standard, calendar-based (drip) irrigation in a warm climate may lead to large but poorly colored berries, deficit irrigation results in medium-size to small berries of good color, regardless of nitrogen supply.

It is clear, then, that the effect of nitrogen on yield and fruit quality depends on the grapegrowing region (climate), soil type and composition, and prevailing weather conditions. In other words, the vineyard site influences our ability to manipulate the yield quality relationship in grapes, using nutrient and water management or other cultural practices.

If our goal is to produce top-quality grapes, it is unwise, and in fact impossible, to use a cookbook approach with a specified amount of fertilizer applied at a specific point in time to each cultivar in every year. The approach to nitrogen nutrition has to be flexible or adaptive, and it depends on the current vine nitrogen status and seasonal conditions.

As vignerons, we should always keep in mind that high yields of excellent quality come from balanced vines with an open canopy and ideal microclimate.

Summary

Effects of nitrogen on yield and quality can be both direct and indirect. The bloom and post-harvest periods are critical for nitrogen supply. A shortage during bloom will result in a loss of potential yield, while a surplus will reduce grape quality.

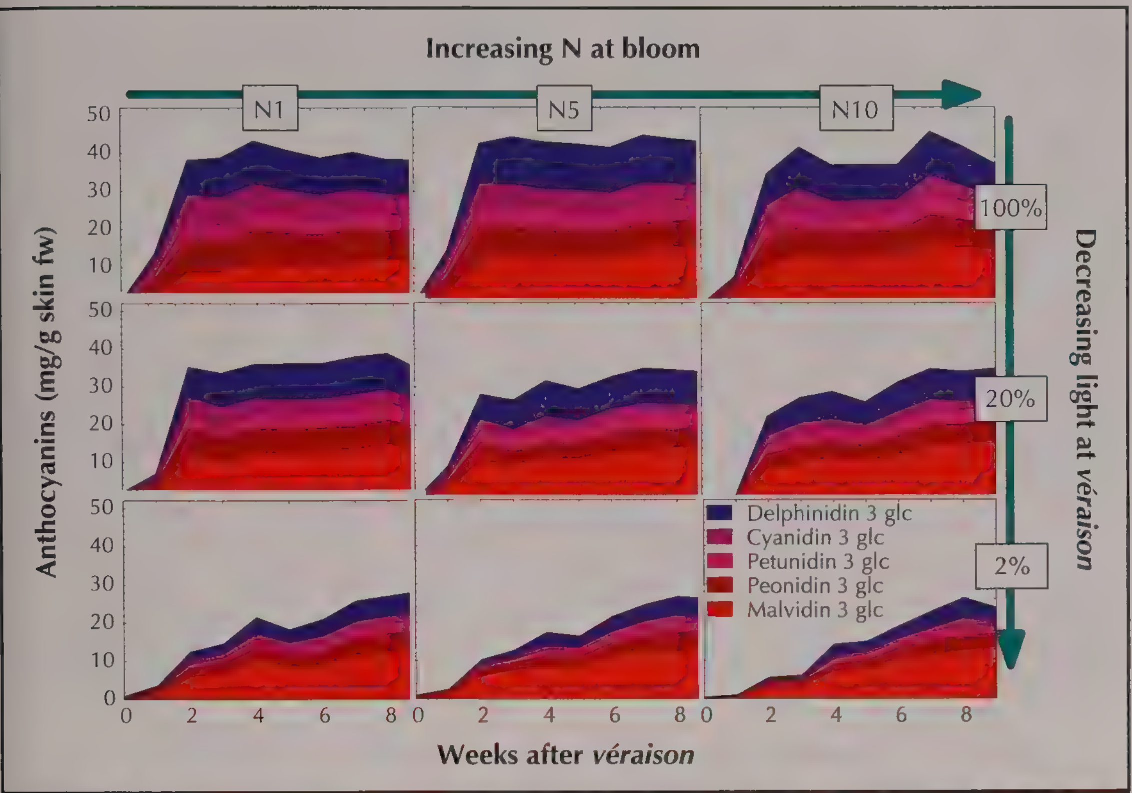

Figure IV: Nitrogen supply during bloom can influence the susceptibility of grapevines to unfavorable environmental conditions later in the season. Note how more nitrogen at bloom results in a less-balanced anthocyanin profile in the grape skins, especially when combined with low-light stress at *véraison*. For explanations see Figure III (modified from Keller and Hrazdina 1998).

Color is among the quality attributes most easily influenced by nitrogen availability, but it is also linked to water supply. Both timing and amount of nitrogen supply depend on vine nitrogen status, climate, weather and soil properties. The goal is to achieve balanced vines with an open canopy and ideal microclimate for high yields and top quality grapes.

The relationship between grape yield and quality is complex and can be influenced by a variety of cultural practices. Nitrogen supply is one of these practices that, along with water supply, is crucial for vine growth, yield formation and fruit quality. Errors made in nitrogen management cannot be corrected by other cultural practices, such as canopy management. PWV

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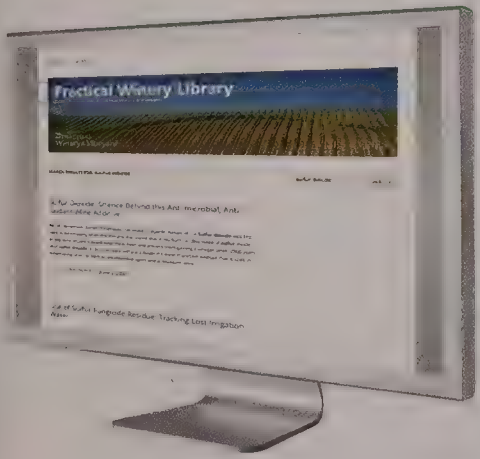

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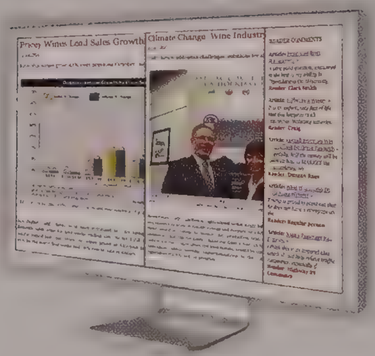

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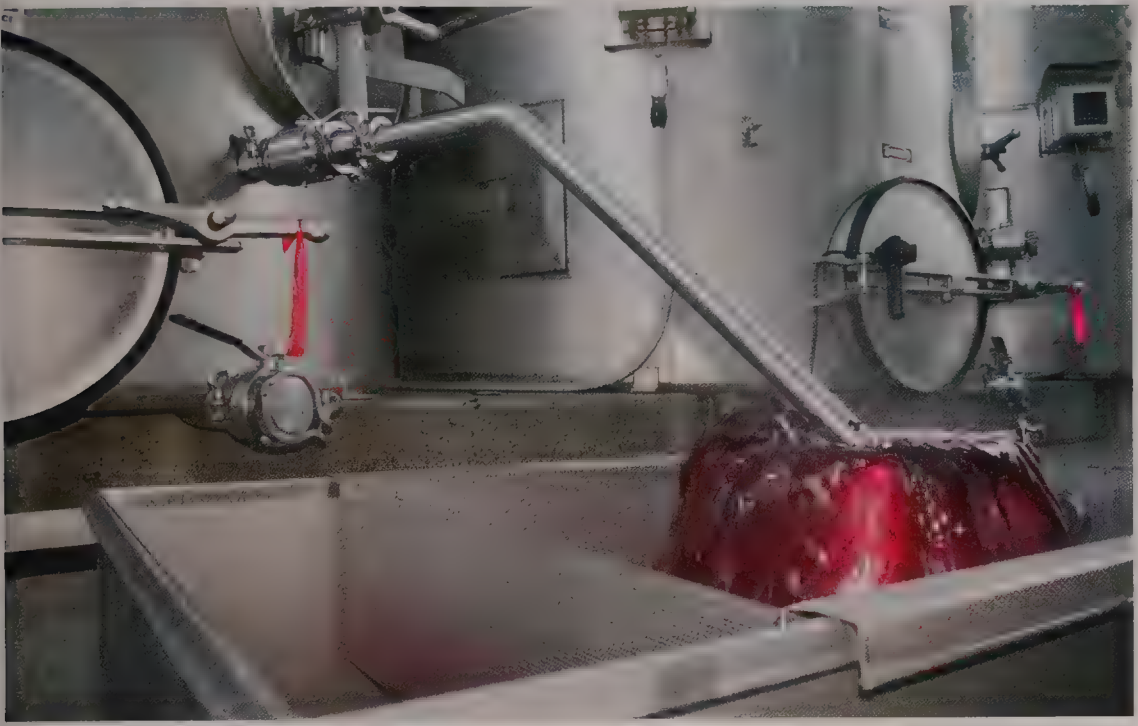

PHOTO BY WENDY DAY, VINE CLIFF WINERY

Aeration across a screen with seed removal during draining of the fermentor.

MERLOT AND CABERNET SAUVIGNON WINES

Impact of délestage

WITH PARTIAL SEED REMOVAL

BY Bruce W. Zoecklein,[1]* Lisa M. Pélanne,[2] Sandy S. Birkenmaier,[3] Karen Reed[4]

*Emeritus professor, former head of the Enology Grape Chemistry Group, Virginia Tech, [2,3]Research Associates, [4]viticultural consultant

Délestage (rack and return) with partial seed removal was compared with manual cap punch down (three years) using Merlot, and with a mechanical punch-down (pigeage) system using Cabernet Sauvignon (one year).

Fermentation reduced the color derived from monomeric pigments and increased polymeric pigment color for all treatments. Délestage wines generally had more color derived from large polymeric pigments compared to cap-punched or pigeage wines. Total glycosides increased during cold soak and fermentation and were in greater concentration in cap-punched Merlot, and similar among Cabernet Sauvignon treatments.

Discrimination testing (triangle difference analysis) demonstrated Merlot wines generally differed in aroma and/or flavor. Cabernet Sauvignon wines differed in both aroma and flavor.

Principal component analysis (PCA) for aroma and flavor demonstrated the variation among control and délestage-produced wines. Analysis of wine volatiles indicated that aromatic esters were generally found in highest concentrations in délestage wines which also demonstrated a decreased level of long chain or fusel alcohols.

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Grape phenol compounds

The color, structure and aftertaste of red wines are mainly derived from the varied and complex impact of phenolic compounds. It is estimated that 50% or less of the total phenolic compounds present in the skins, seeds and flesh of grapes can be extracted during conventional winemaking.[12,36]

The level of extraction depends on various factors, including fruit maturity, duration of skin contact, temperature, ethanol concentration,[20] and vinification practices, including cap management techniques.[7,16,22,31] Therefore, understanding the quantitative and qualitative influences processing has on grape and wine phenolic compounds is important in premium wine production.

Monomeric and polymeric flavan-3-ols comprise the majority of phenolic constituents in red wines,[30] being extracted from the skins and outer seed coat during fermentation.[38] Polymeric flavan-3-ols, referred to as proanthocyanidins or condensed tannins, arise either by addition of intermediates from flavan-3,4-diols to flavan-3-ol monomers, or by acetaldehyde-induced polymerization.[8,35]

Grape seeds differ from skins in that seed proanthocyanidins contain greater levels of monomeric flavan-3-ols, and those esterified to gallic acid.[5,25,32] Additionally, seed proanthocyanidins generally have a lower degree of polymerization (dp) than those found in skins, and no trihydroxylation of the B-ring.[9] Proanthocyanidins are reactive molecules that may form complex species thought to impact wine sensory features.

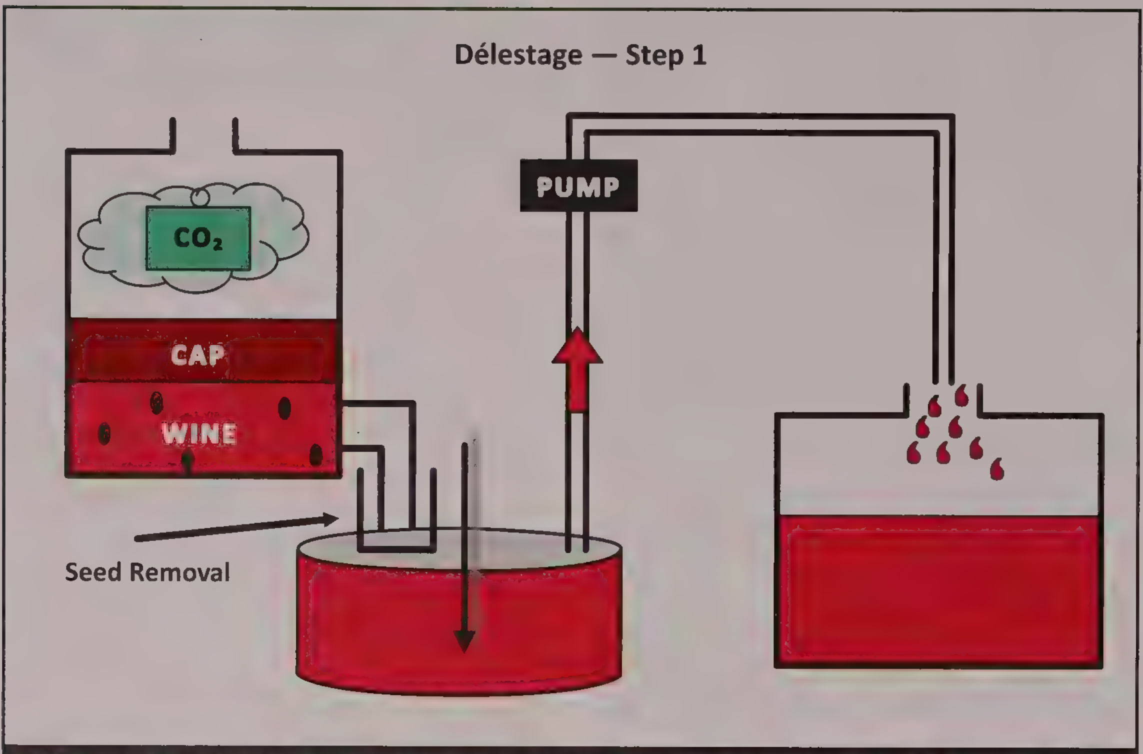
Monomeric and polymeric flavan-3-ols induce both astringent and bitter mouth sensations. S. Vidal *et al.* demonstrated that overall astringency increased with increases in dp.[38] Additionally, they reported that galloylation increased tannin coarseness, while trihydroxylation of the B-ring decreased coarseness.

Tannins in the skins and seeds can combine with anthocyanidin glycosides (anthocyanins) to form polymeric pigments.[36] These pigments are believed to be formed by condensation products of malvidin-3-glucoside and various procyanidins created through acetyl bridges.[9,26] Anthocyanin-tannin complexes can be produced by binding between the C-4 of the flavylium salt and the C-8 of catechin.[21,27]

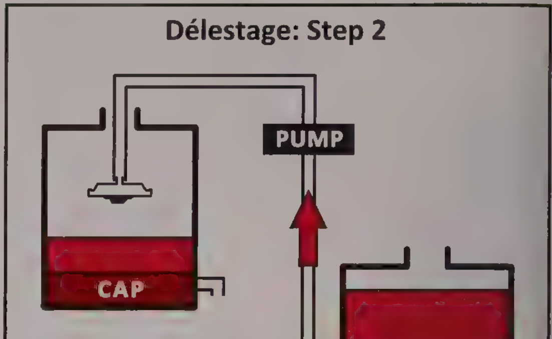
D.O. Adams *et al.* reported extractable seed tannins in Syrah grapes declined by about half from véraison to harvest, and were about three times greater than skin tannin concentrations.[2] Grape skin phenols are more easily extracted during fermentation than those of seeds and stems.[18]

Although skins contain a lower concentration of total and polymeric phenols than seeds,[13] they may be the primary source of polymeric phenols in wine.[14] For the first five to seven days of fermentation, phenolic compounds are extracted mainly from skins, followed by extraction from seeds.[23]

Several reports have suggested that seeds contribute significant concentrations of proanthocyanidins to wines,[15,29] while others have reported the seed contribution to be limited.[4,24,39] These contradictory observations may be the result of differences in cultivar, fruit maturity, and



Délestage is a rack-and-return process modified to deport seeds, illustrated here. Fermenting juice was deported from a bottom valve through a dejuicing sleeve with holes 1/32-inch in diameter. Seeds were retained within the sleeve, and the deported juice was pumped to a separate tank while the cap was allowed to drain. Process was conducted once per day until the completion of fermentation or dejuicing, depending on the particular trial.



(Adapted from Delteil [1998])

Juice was brought back to top of the fermentation tank using a cap irrigation system. Délestage, therefore, involves two main features which are impacting the tannin profile of the wine (and therefore the structural/textural qualities):
1) removal of seeds could reduce the contribution of immature phenols;
2) oxidative polymerization could result in partial reduction of monomeric pigments as they are incorporated into large polymeric pigments.
(For more details see www.icv.fr/kiosqueuk/procedur/delestage.htm or www.vtwines.info/enologynotes/onlinepublications)

winemaking style.

For example, duration of maceration primarily influences the extraction of phenolic compounds from the seeds,[40] while fermentation temperature appears to be a primary factor influencing extraction from skins.[23]

Délestage

Délestage, or rack and return, is a maceration technique designed to help optimize the exchange between the liquid and solid phase by emptying the fermentation vessel of liquid during aeration.

Following several hours of cap-draining, the liquid is gently pumped over, or returned, to the cap. This procedure is designed to help oxygenate, while minimizing mechanical grinding of the skins, seeds and stems (Dominique Delteil, personal communication, 2003). Only if this procedure is performed to minimize mechanical extraction can délestage be considered a gentle cap management strategy. Temperature management and control when délestage is conducted may also be an important practical issue.

This study evaluated délestage in conjunction with partial seed removal, to determine the impact on Merlot wine composition for three seasons and on Cabernet Sauvignon for one season.

Materials and Methods

Merlot fruit (approximately 8,500 kg), grown in central Virginia, was hand-harvested in each of three years at a minimum of 21.0° Brix (a common soluble solids concentration for Merlot grown in central Virginia). Fruit was immediately destemmed, crushed and divided into six equal-weight (1,416 kg) replicates. Must fermentable nitrogen levels were measured, and adjusted to 250 mg/L adding either Fermaid K or Superfood™. Sulfur dioxide (30 mg/L) was added at crush to each lot.

Each must was given a cold maceration (cold soak) period of 24 hours at 10° C, prior to fermentation. D-254™ yeast was hydrated, microscopically examined for budding, viability and purity, cooled to within 3° C of the must temperature, and added to each lot (24 g dry yeast/100 L).

The six equal-weight lots were randomly assigned to treatments consisting of 1) control, conventional fermentation, with cap manually punched down two times per day, or 2) délestage, consisting of a rack and return procedure with seed removal conducted once per day until dryness, as follows.

Following cap rise, fermenting juice was drained from a bottom valve through an external cylindrical dejuicing sleeve (2.39 mm diameter holes) into a stainless steel vat. Seeds were retained within the sleeve.

The juice was pumped to a separate tank while the dejuiced cap was allowed to drain freely for two hours. Juice was then returned to the top of the cap via a tank cap irrigator, using deflection plates to minimize skin breakage. The separated seeds were drained free of liquid, weighed and discarded.

Treatment and control vessels averaged filled height-to-diameter ratios of 0.64 and 0.75 for the délestage and conventional fermentations, respectively.

Fermentations were conducted at an average liquid temperature of 28° C (range 26° to 35° C) and an average cap temperature of 30° C (range 28° to 37° C) in 1,000-L capacity vessels. Pressing was performed at dryness (2.0 g/L reducing sugar) using a tank press to 1 bar. Free-run and press-run wines were combined.

Cabernet Sauvignon fruit (18,144 kg) grown in northern Virginia was hand-harvested at 23° Brix, immediately destemmed, crushed, sulfur dioxide (30 mg/L) added, fermentable nitrogen levels were measured and adjusted, and divided into treatment lots (as described above). Musts were given a cold maceration (cold soak) period of 48 hours at 10° C prior to fermentation, and yeasted (as described above).

Treatments consisted of 1) fermentation using 10,000-L mechanical pigeage, or 2) délestage conducted in similar size and shape static stainless steel fermentation tanks (fill height to diameter ratio, 1:1).

Pigeage consisted of punching the cap three times daily, 10 minutes per punch, with punching consisting of cycles of one minute down and 30 seconds up. Délestage was conducted daily as described above with the following exception: liquid was drained onto a flat tray (0.75 x

Table I: Effect of manual cap punching (control) and délestage on Merlot wine chemistry for three seasons.

	Season 1		Season 2		Season 3	
	Control	Délestage	Control	Délestage	Control	Délestage
Alcohol % (v/v)	12.8a[a]	12.7a	11.5a	11.7a	13.1a	13.1a
TA (g/L)	6.57a	6.70a	6.20a	6.38a	4.85a	4.88a
Tartaric Acid (g/L)	2.21a	1.97a	3.06a	3.41a	1.50a	1.74a
Malic Acid (g/L)	trace	trace	trace	trace	trace	trace
Lactic Acid (g/L)	3.15a	2.23a	3.35a	4.07a	3.87a	2.44a
pH	3.60a	3.66a	3.65a	3.66a	3.87a	3.91a
Total Tannin (mg CE/L)	191.6a	173.0b	177a	150b	197.5a	171.1b
Total Phenol (AU^{280})	59.8a	58.3a	43.1a	37.6b	40.1a	37.0b
Total Anthocyanin (AU^{20}–AU^{SO_2})	ND[b]	ND	3.89a	3.04b	2.37a	2.21b
$AU^{420+520}$	8.23a	6.92b	8.21a	7.82a	8.87a	7.64a
$AU^{420/520}$	0.793a	0.780a	0.794a	0.789b	0.575b	0.585a

[a] Different letters within rows and years denote significant difference ($p \leq 0.05$) of treatment means; [b] ND = not determined; n = 3.

1.2 m) with a screen (2.39 mm diameter holes).

Fermentations were conducted at an average liquid temperature of 27° C (range 26° to 33° C) and an average cap temperature of 30° C (range 28° to 34° C). Mechanical punching and délestage were conducted for seven days.

Pressing was performed post-dryness (2.0 g/L reducing sugar), 22 days following the beginning of fermentation, with a 5,000-L tank press, by allowing free drainage for one hour, followed by pressing to one bar. Free-run and press-run wines were not combined.

Chemical analysis

General fruit, must and wine chemistries were conducted as described by B. Zoecklein *et al.*[43] HPLC analysis was conducted 18 months post-fermentation on selected phenols in finished aged wines described by S. Price *et al.*[19]

Total tannins (catechin equivalents), and the percentage of color from monomeric pigments (MP), small polymeric pigments (SPP), and large polymeric pigments (LPP) was estimated using the procedures of Adams and Harbertson,[1] and Harbertson *et al.*[11] The concentration of total glycosides was estimated by the analysis of glycosyl-glucose in thawed samples as described by P.J. Williams *et al.*,[41] and modified by R.S. Whiton and B.W. Zoecklein.[40] Analysis of phenol-free glycosides was conducted as described by B. Zoecklein *et al.*[44]

Wine volatiles were analyzed using solid-phase micro-extraction and gas chromatography-mass spectrophotometry, as described by B. Zoecklein *et al.*[43] using a model 5890 GC, model 5972 mass selective detector, and a Carbowax 65-µm fiber.

Sensory analysis

Discrimination testing was performed on pooled wine replicates of Merlot and Cabernet Sauvignon, using triangle difference comparison described by M. Meilgaard *et al.*[17] The wines were evaluated six to eight months post-fermentation in the Virginia Tech wine sensory laboratory, under controlled conditions that included red lighting to help eliminate color bias.

Panel membership required regular wine consumption (at least one glass per week) and attendance at two informational sessions where the methodology of evaluation was described. Evaluation was done based on olfactory (aroma) and retronasal aroma and mouthfeel (referred to as flavor). Evaluations of aroma and flavor occurred at different times.

Descriptive analysis was performed nine months post-fermentation on non-pooled Cabernet Sauvignon wine treatment replicates, using 11 trained panelists as described by M. Meilgaard *et al.*[17] Panel members evaluated three replications of the two products (pigeage and délestage) six times.

Panelists had one to 10 years experience in descriptive or consensus sensory analysis. A list of descriptors was developed from three pre-evaluation training sessions with standards used for training prepared as reported by B. Zoecklein *et al.*[43]

Merlot results

The Merlot fruit averaged 21.5° Brix, 3.7 pH and 5.62 TA for the three years, typical of the region. Berries averaged 1.18 g, with 2.4 seeds, for the three years. In years two and three, Merlot fruit monomeric pigments (MP) resulted in an average of 70.5%, while small polymeric pigments (SPP) 19.7% and large polymeric pigments (LPP) 9.8% of the total color.

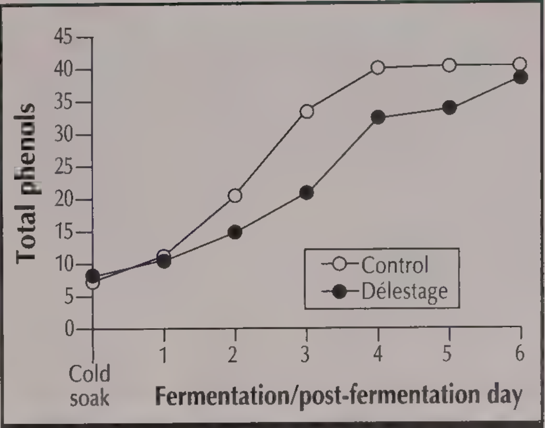


Figure I. Effect of cold soak, fermentation, and post-fermentation on total phenols of control (cap punched) and délestage-produced Merlot wines in season 3; n = 3.

By the end of délestage-treated fermentations, an average 25% of seeds had been removed each year. Fermentation rates were similar among treatments. Total phenols, estimated by the absorbance at 280 nm, increased linearly from crush until dejuicing for both délestage and control wines (Figure I). At day-six (dryness), control lots had a total phenol concentration slightly greater (7.7%) than the délestage (typical of this study).

The percentage of color derived from the monomeric pigments was greater in the fruit than the wine, while the percentage of color from polymeric pigment forms showed the opposite trend.

Merlot délestage and control wines showed slight differences in the percentage of color from the different pigment sources. Délestage wines produced over three years averaged 4.8% lower color derived from monomeric pigments, 1.4% higher from SPP and 4.5% higher color from LPP, compared to control wines (Figure II).

Following fermentation, control and délestage-produced Merlot wines did not differ in alcohol percent (v/v), TA, tartaric, malic, and lactic acids, or pH (Table I).

The total tannin concentration was greater in the control wines upon completion of fermentation each year. The total phenol estimations demonstrated a higher concentration in control wines in two of the three years. Total anthocyanins were higher in the control wines in the two years measured, while absorbance at 420 nm + 540 nm, and 420 nm/520 nm, did not demonstrate consistent patterns between délestage and control wines.

Table II provides the concentration of selected phenolic compounds on aged Merlot determined by HPLC analysis. Significant differences among treatments were not observed. Catechin and epicatechin concentrations averaged 37 and 26 mg/L for the control and délestage-produced wines, respectively.

Merlot total glycosides increased by day-2, the first day of fermentation (Table III). By the completion of fermentation (dejuicing), the total glycoside concentration had increased by an average of 388% and 296% for the control and délestage wines, respectively.

At dejuicing, the total glycoside concentration was greater in the control wines. Phenol-free glycosides increased by day-two. They generally declined by the end of fermentation, and were in greater concentration in the délestage-produced wines at dejuicing.

Results of discrimination sensory analysis suggested that Merlot wines were perceived to differ in aroma and/or flavor in two of three years.

Cabernet Sauvignon results

Cabernet Sauvignon must underwent cold maceration for 48 hours, prior to yeast addition. The effect of fermentation on reducing sugar concentration and percent alcohol (v/v) at various sampling periods was determined by comparing one fermentation vessel each of délestage- and pigeage-produced Cabernet Sauvignon (Figure III).

While the fermentation rates were generally similar between treatments, some differences in the wines were noted. There were no differences in alcohol percent (v/v), TA, pH, tartaric, malic or lactic acids, between pigeage and délestage-produced wines (Table IV).

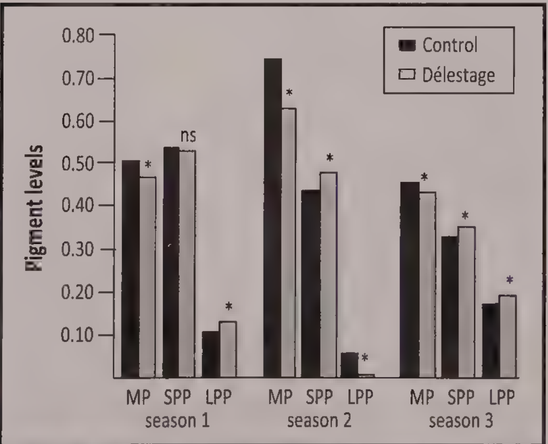


Figure II. Effect of control (cap punched) and délestage on Merlot — percent color derived from monomeric pigments (MP), small polymeric pigments (SPP), and large polymeric pigments (LPP) for three seasons; n = 3.

Table II: Mean values of HPLS analysis of phenolic profiles of aged Merlot wines (for three seasons), and Cabernet Sauvignon wine (produced one season). Significant differences were not observed at $p \leq 0.05$.

	Merlot		Cabernet Sauvignon	
	Control	Délestage	Pigeage	Délestage
Gallic Acid (mg/L)	22[a]	21	55	37
Catechin (mg/L)	23	17	49	39
Epicatechin (mg/L)	14	9	29	16
Caftaric Acid (mg/L)	11	11	<1	6
Caffeic Acid (mg/L)	14	12	16	20
Quercetin (mg/L)	8	4	3	3
Malvidin Glucoside (mg/L)	30	13	20	21
Polymeric Anthocyanins (mg/L)	34	36	36	41
Total Anthocyanins (mg/L)	100	83	71	61
Monomeric Anthocyanins (mg/L)	50	28	25	30

[a] n = 3

Total tannin, total phenols and total anthocyanins were greater in pigeage-produced wines. Differences in absorbance at 420 nm + 520 nm, and 420 nm/520 nm, were noted between délestage and pigeage. Table II provides the concentration of selected phenol compounds on aged Cabernet Sauvignon. Significant differences among treatments were not observed.

Tannin concentrations remained stable until active fermentation, then increased and were higher in pigeage-produced wines at most sample periods (Figure IV). Total phenols (AU 280) increased for both treatments during pre-fermentation maceration, and significantly during fermentation (Figure V).

At dejuicing, the total phenol concentration in the press wines averaged 14.5% and 9.8% higher than free run for délestage and pigeage wines, respectively (data not shown). At the completion of fermentation, free-run pigeage-produced wines had higher absorbance at 420 nm + 520 nm, and lower 420 nm/520 nm absorbance than délestage wine (Table IV).

During the cold soak period, the per-

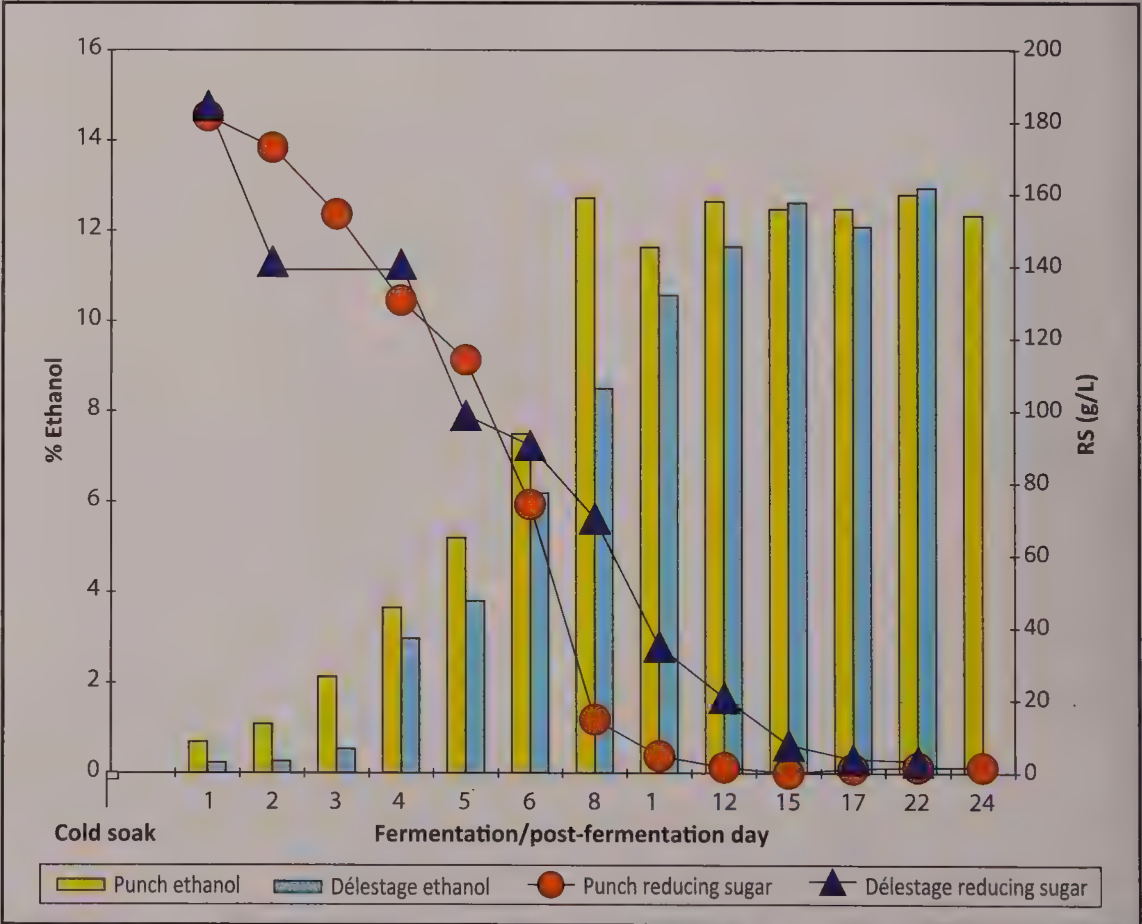


Figure III. Effect of mechanical punch (pigeage) and délestage on Cabernet Sauvignon ethanol production and reduced sugar decrease; n = 3.

Table III: Effect of manual cap punch (control) and délestage on total (TGG) and phenol-free (PFGG) Merlot glycosides for two seasons.

Sample		TGG (µM)		PFGG (µM)	
		Control	Délestage	Control	Délestage
Season 2	Post-Cold Soak	386b^a	435a	157b	191a
	Day 2	1513a	1156b	180b	264a
	Dejuice	2068a	1866b	135b	194a
Season 3	Post-Cold Soak	360b	394a	144a	152a
	Day 2	1260a	1279a	141a	127a
	Dejuice	1583a	1433b	120b	134a

^{a}Different letters within rows indicate significant difference ($p \leq 0.05$) of treatment means; n = 3.

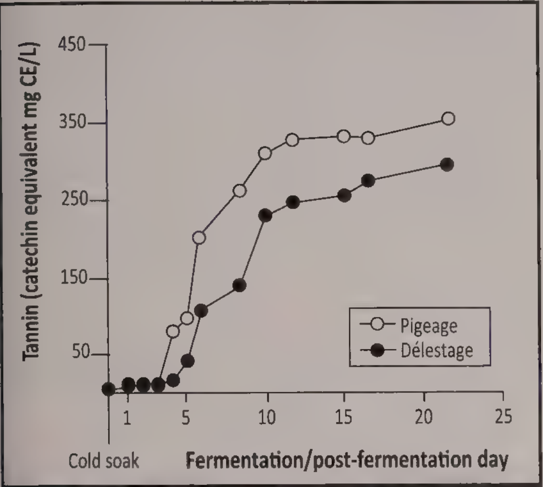


Figure IV. Effect of pigeage and délestage on Cabernet Sauvignon — tannin concentration during cold soak, fermentation, and post-fermentation; n = 3.

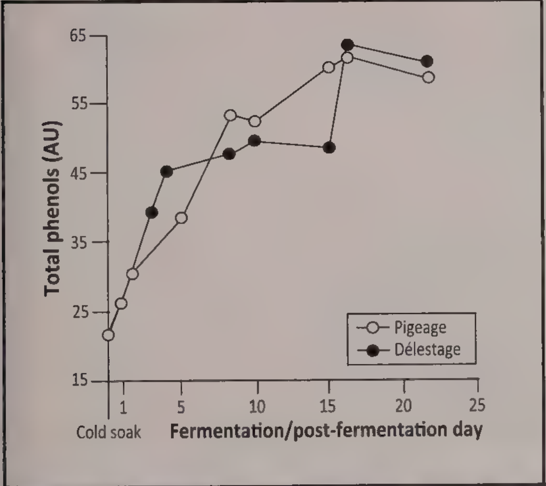


Figure V. Effect of pigeage and délestage on Cabernet Sauvignon — total phenols during cold soak, fermentation, and post-fermentation; n = 3.

centage of color from monomeric anthocyanins declined dramatically in the juice, then declined or remained constant for the first three days of fermentation (Figure VI).

By sampling on day-10 (completion of alcoholic fermentation), the percentage of monomeric pigments had declined for both treatments. At dejuicing, day-22, the percentage of color from monomeric pigments in the pigeage free-run wine averaged 33% higher than the délestage wine.

Press wines showed a similar trend (data not shown). The percentage of color from small polymeric pigments increased during the cold soak period, remained or declined during the first five days of fermentation for both treatments, then increased slightly (Figure VII).

The percentage of color from large polymeric pigments increased during cold soak and fermentation for both pigeage and délestage treatments, and was slightly higher in the délestage wines at dejuicing (Figure VIII).

Post-fermentation, free-run Cabernet Sauvignon délestage and pigeage wines demonstrated 34.6% compared to 43.5% color from monomeric pigments, 53.8% compared to 49.6% color from SPP and 11.6% compared to 6.9% color from LPP (Table IV), respectively.

Following cold soak, total glycoside concentration was greater in the pigeage than délestage tanks by an average of 49% (Table V). Total glycosides increased during fermentation (cold soak to day-10) for both treatments. By the completion of fermentation (day-10) and at dejuicing, total glycoside concentrations were similar in pigeage and délestage wines. Phenol-free glycosides were in higher concentrations in pigeage wines post-cold soak and at dejuicing.

Discrimination sensory analysis of Cabernet Sauvignon délestage- and pigeage-produced wines indicated differences in aroma and flavor. The principal component analysis (PCA) for aroma indicated variation among treatment replicates that accounted for 59% of the variance (Figure IX). The first and second principal component analysis of flavor accounted for 63% of the variance (Figure X).

Discussion

A relatively high concentration of extract-

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able seed tannins has been shown to negatively impact wine quality in Virginia and other wine-producing regions. The Merlot study was conducted using 1,416 kg lots, and seed removal in conjunction with délestage, to help improve red wine mouthfeel. Due to logistical limitations, including the necessity for replications, wines were not produced by délestage alone, without seed removal.

Table IV: Effect of pigeage and délestage on Cabernet Sauvignon wine chemistry, and average percentage of color derived from monomeric pigments (MP), small polymeric pigments (SPP), and large polymeric pigments (LPP).

	Pigeage	Délestage
% Alcohol (v/v)	12.4a[a]	12.5a
TA (g/L)	5.19a	5.01a
Tartaric Acid (g/L)	1.36a	1.32a
Malic Acid (g/L)	0.52a	0.52a
Lactic Acid (g/L)	4.12a	3.72a
pH	3.96a	4.01a
Total Tannin (mg CE/L)	337.8a	294.5b
Total Phenols (AU^{280})	65.2a	58.0b
Total Anthocyanin (AU^{20}–AU^{SO_2})	2.65a	1.67b
$AU^{420+520}$	0.616a	0.608b
$AU^{420/520}$	0.77b	0.81a
Monomeric Pigment (%)	43.5	34.6
Small Polymeric Pigment (%)	49.6	53.8
Large Polymeric Pigment (%)	6.9	11.6

[a] Different letters within rows denote significant difference ($p \leq 0.05$) of treatment means; n = 3.

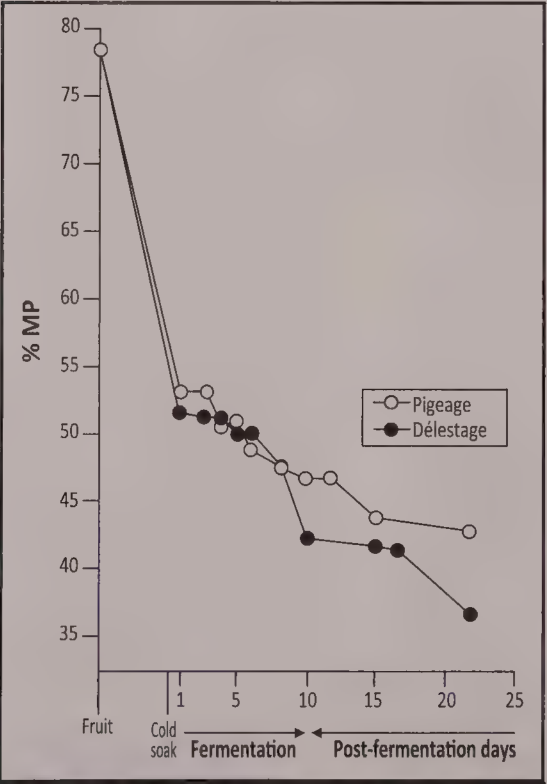

Figure VI. Effect of pigeage and délestage on Cabernet Sauvignon — monomeric pigments (MP) as a percentage of total color during cold soak, fermentation, and post-fermentation; n = 3.

The majority of the seeds removed (average 25%) were removed in the first few days of fermentation, possibly contributing to the lower total tannin concentration frequently observed in délestage-produced wines. Tannin levels generally remained stable in the must until active fermentation, then increased significantly.

V. Singleton and P. Draper demonstrated that fermentation for 90 hours resulted in extraction of 65% of the available seed tannins, while 180 hours resulted in the extraction of 70%.[28] Seed tannins comprise approximately 60% of the total phenols in conventionally-produced red wines,[28] with nearly half of the extractable catechins and oligomeric proanthocyanidins in grape seeds transferred into wine.[37]

V. Kovac *et al.* added seeds during fermentation (6% of the weight of the fruit) and noted a doubling in the concentration of catechins and proanthocyanidins in the fermented wine.[15] For the Merlot wines, about 1.1% of the weight of the fruit was removed as seeds during délestage. A. Bosso *et al.* compared pump over with délestage, using Montepulciano d'Abruzzo, and found that pump over produced wines higher in anthocyanins, polymeric pigments and tannins.[3]

In this study, délestage wines contained a lower tannin concentration than controls (manual cap punch down or pigeage), possibly due to limited extraction and seed removal in délestage treatments. However, HPLC analysis of aged wines did not demonstrate statistical differences in selected phenols, including those associated with seeds, such as catechin and epicatechin.

Phenol extraction from seeds is dependent, in part, on the degree of seed oxidation or maturity.[9] Délestage can allow fermenting juice to percolate through the cap, providing an exchange that may minimize particulate extraction from the cap (Dominique Delteil, 2003, personal communication).

Although not measured in this study, it is possible that délestage reduced the concentration of non-soluble solids, thereby aiding in reduction of total phenols, including skin tannins. Total anthocyanins were frequently in greater concentrations in conventional- and pigeage-produced wines, compared to délestage, possibly suggesting greater extraction.

The higher concentration of total glycosides noted in manual cap-punched Merlot wines may also indicate increased extraction, although there were no differences in total glycosides in the Cabernet Sauvignon produced by pigeage and délestage.

Formation of polymeric pigments is important due to their contribution to color stability. It has been demonstrated that, after only a few years of ageing, the vast majority of color is due to polymeric pigments, with a small concentration of monomeric anthocyanins remaining.[33]

Analysis of the fruit demonstrated a relatively high percentage of color from monomeric pigments compared to LPP, consistent with D. Adams *et al.*,[2] and J. Harbertson *et al.*[11]

In the second and third years, the Merlot fruit LPP averaged 9.8% of the color, while corresponding wines averaged 18.5% color from LPP. The increase in percentage of wine color from LPP, compared to the fruit, appeared to parallel a decrease in the percentage of color from monomeric anthocyanins in the wine.

It is generally assumed that formation of polymeric pigments is the result of relatively slow, post-fermentation reactions.[34] J. Eglinton however, demonstrated that fermenting yeast cells and their metabolites are actively involved in condensation reactions with tannins and anthocyanins, suggesting polymeric pigment formation during fermentation.[6]

In this study, it must be noted that the analyses of the percentage of color from MP, SPP and LPP (monomeric, small polymeric and large polymeric pigments) are estimations. For example, while not impacted by the phenolic matrix,[11] monomeric anthocyanins at the pH of the assay are largely in the leuco- or colorless form.

The percentage of Cabernet Sauvignon color from monomeric pigments declined during fermentation for both treatments, by an average of 25%. A. Zimman and A. Waterhouse demonstrated that a significant percentage of the loss of monomeric pigments could be due to association with grape solids.[42] Therefore, it is possible that a cap management technique that impacts the non-soluble solids level could impact monomeric anthocyanins.

The higher percentage of color from monomeric pigments in pigeage wines at the end of fermentation may reflect increased fruit extraction. Cabernet Sauvignon color from SPP increased during

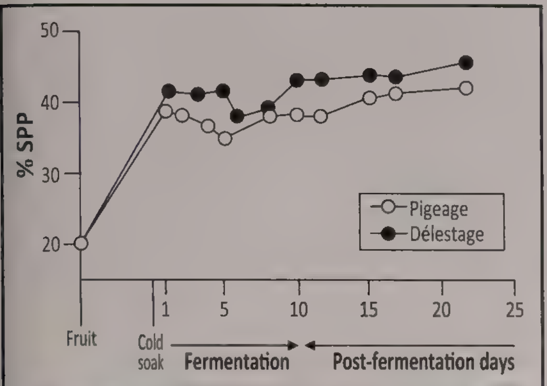

Figure VII. Effect of pigeage and délestage on Cabernet Sauvignon — small polymeric pigments (SPP) as a percentage of total color during cold soak, fermentation, and post-fermentation; n = 3.

cold soak, and appeared to increase only slightly from the beginning of fermentation to dejuicing (average 7.8%). The percentage of color from LPP increased during fermentation by approximately 150%.

The Cabernet Sauvignon LPP-to-SPP ratio, as percent of color, ranged from 0.11 during cold soak to 0.37 at dejuicing. The SPP would be expected to contain pigment dimers and trimers formed by acetaldehyde crosslinking of anthocyanin and flavan-3-ols.[28] The LPP fraction likely contains anthocyanins that have

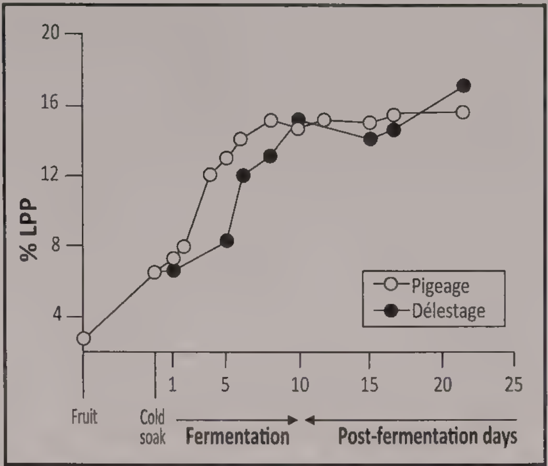

Figure VIII. Effect of pigeage and délestage on Cabernet Sauvignon — large polymeric pigments (LPP) as a percentage of total color during cold soak, fermentation, and post-fermentation; n = 3.

reacted directly with polymeric flavan-3-ols, or by acetaldehyde crosslinks, to form polymeric pigments large enough to precipitate with BSA in the assay.

Phenol-free glycosides were in larger concentration in Merlot, but not Cabernet Sauvignon délestage-produced wines. The analysis of phenol-free glycosides includes all but shikimic acid metabolites. This analysis may be a better approximation of the glycosidically-derived aroma/flavor pool than the total

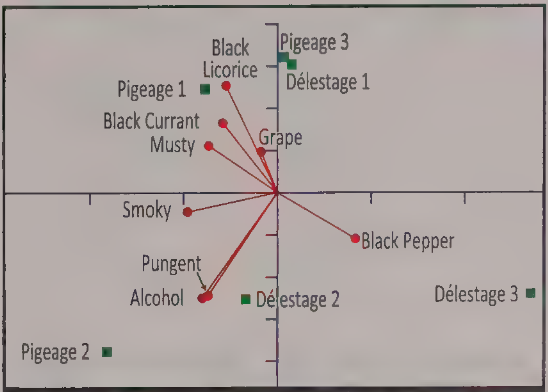

Figure IX. Projection of aroma attributes on principal components I and 2 for Cabernet Sauvignon pigeage- and délestage-produced wines; n=II.

glycosides assay.

Discrimination sensory analysis on pooled treatment replications indicated differences in aroma and flavor among Merlot and Cabernet Sauvignon délestage and control wines. PCA analysis of Cabernet Sauvignon treatment replications demonstrated differences between délestage and pigeage wines, and among replications of the same treatment.

It is evident that délestage wine-1 and pigeage wine-3 have similar aroma and flavor profiles. While treatments were dejuiced each day at the same Brix, individual replicate variation occurred, pos-

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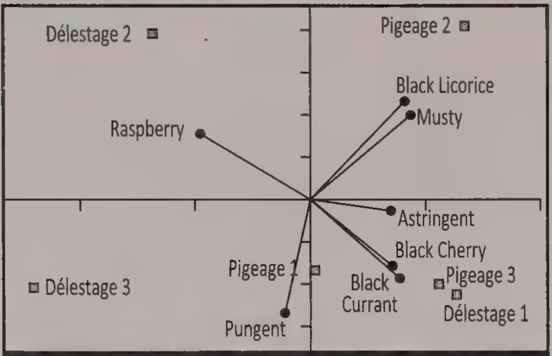

Figure X. Projection of flavor attributes on principal components I and 2 for Cabernet Sauvignon pigeage- and délestage-produced wines; n=II.

sibly as a result of the degree of seed removal, pomace drain time and/or oxygen exposure. With the exception of replicate-one, délestage wines were characterized by pungent black pepper aromas and pungent raspberry flavors.

Volatile components were determined by head-space solid phase micro extraction and quantified using gas chromatography/mass spectrometry (GCMS). The overall trend across three vintages and two cultivars is represented by Table V1. The acetate and fatty acid esters were found to parallel ethanol formation. The higher boiling point, more aromatic esters were generally found in highest concentrations in the delestage wines which also demonstrated a decreased level of long chain or fusel alcohols.

Conclusion

An important winemaking goal is to be able to customize maceration methods, predicated on fruit composition and desired outcome. This study evaluated the impact of a cap management technique in conjunction with seed removal.

Given the large variability in fruit composition, the response to a particular maceration technique may be variable. Délestage with partial seed removal appeared to slightly modify the percentage of color derived from monomeric and large polymeric pigments. The result of discriminatory sensory analysis generally suggested differences in aroma and flavor between délestage and control wines. Sensory results were supported by difference noted in aroma/flavor compounds. PWV

Table V: Effect of mechanical pigeage (P) and délestage (D) on total (TGG) and phenol-free (PFGG) Cabernet Sauvignon glycosides.

	TGG (µM)		PFGG (µM)	
Sample	P	D	P	D
Post-Cold Soak	986a[a]	662b	189a	98b
Day 10	1441a	1505a	139b	154a
Dejuice	1470a	1470a	113a	94b

[a] Different letters within rows and assays indicate significant difference ($p \leq 0.05$) of treatment means; n = 3.

Table VI: Volatile compounds found to be significantly higher in Delestage and control Merlot and Cabernet Sauvignon wines

Délestage	Control
Ethylacetate	2-Methylpropanol
Ethyloctanoate	3-Methylbutanol
Acetic Acid	Ethylhexanoate
Ethyldecanoate	n-Hexanol
Diethylsuccinate	
Phenylethylacetate	
Benzyl Alcohol	
Octanoic Acid	
Decanoic Acid	

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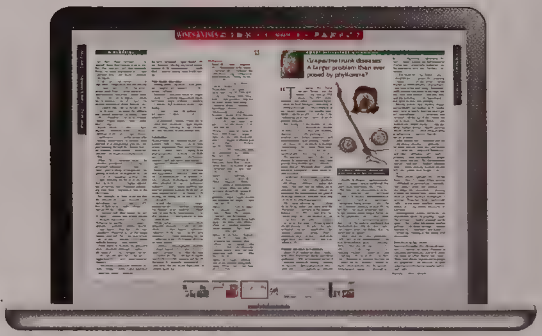
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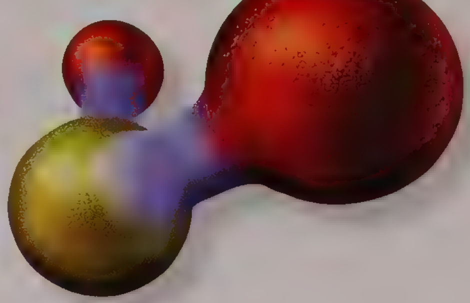
SULFUR DIOXIDE

The science behind this anti-microbial, anti-oxidant wine additive



BY **Pat Henderson,** Senior Winemaker,
Kenwood Vineyards, Kenwood, Calif.,
Author of *About Wine*

Sulfur dioxide was first used in winemaking when the Romans discovered that if you burn candles made of sulfur inside empty wine vessels it would keep them fresh and prevent them gaining a vinegar smell. 2,000 years later sulfur dioxide, or SO_2, still remains without a doubt the most important additive that is used in winemaking.

Used as both an antimicrobial agent and antioxidant, winemakers find it indispensable to preserve wine quality and freshness. However, if used improperly, the effects can be just as adverse as they can be beneficial.

All wines benefit from tender care, whether one is crushing, racking, or bottling, the gentlest method of accomplishing a task is usually the most successful. Just as it is with any of the other tools and techniques a vintner may use, the addition of sulfur dioxide works best when enough is added at the proper time to accomplish the desired result without adding too much and adversely affecting wine quality.

The amount and timing of sulfur dioxide additions depends on the style of wine that is being made and the composition of the wine to which it is being added. While it is possible to make wine without adding sulfur dioxide, you cannot make wine that contains no sulfur dioxide at all. This is because yeast produce a small amount, about 10 parts per million, during fermentation.

When adding sulfur dioxide to must or wine it is important to consider the stage of winemaking that it is in, such as fermentation, ageing or prebottling. You should also consider what the status of the malolactic fermentation is and whether you want to encourage or discourage it, and how turbid or clean the wine is, and how long it will be before the wine is consumed.

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Because of these variables and the variation in winemaking styles, every winemaker or winemaking text is likely to have a different answer to the question: "how much sulfur dioxide should be added?" This confusion can be frustrating to novice vintners but it illustrates one important point; there are few absolute rules of winemaking and depending on the situation and the type of wine that is being made there are many options.

> Every winemaker or winemaking text is likely to have a different answer to the question: "How much sulfur dioxide should be added?"

Additionally, since there is no "one size fits all" answer on the use of sulfur dioxide in wine, it is important to have an understanding of the chemistry of sulfur dioxide and how it reacts in a given wine before it can be used properly.

The subject of chemistry can be daunting for those who have not studied it since high school and the chemistry of sulfur dioxide in wine is no exception. Because of this, some winemakers have only a basic knowledge of how sulfur dioxide reacts in wine and the different forms that it takes.

Chemistry of sulfur dioxide

Sulfur is an element found on the periodic table. In its pure form, it can be dusted or sprayed on grapevines during the growing season to prevent rot and mildew from developing. If sulfur is **oxidized**, it forms sulfur dioxide or SO_2.

Oxidation is the term used by chemists to describe when an element or compound, such as sulfur, loses electrons. While oxidation reactions do not necessarily have to have the presence of oxygen to occur, they often do because when oxygen reacts with an element or compound it readily accepts electrons.

The burning of sulfur in the air oxidizes it and produces SO_2 in the chemical reaction: $S + O_2 = SO_2$.

Sulfur dioxide gas has a sharp pungent aroma that smells like a burnt match, this is hardly surprising because match heads contain sulfur and when they ignite, they release SO_2.

In a chemical reaction where sulfur gains electrons it is said to be reduced. Compounds that are made up of reduced sulfur are called **sulfides**. Sulfide compounds are characterized by a strong unpleasant odor.

Hydrogen disulfide (H_2S), is a wine spoilage compound that has an aroma that smells like rotten eggs. It can have several causes, but it is most frequently a result of residual sulfur dust present on grapes when they are harvested, being reduced by yeast to H_2S during fermentation.

Hydrogen disulfide can also be caused by a shortage of yeast nutrients during fermentation. H_2S can undergo further chemical reaction to form compounds called mercaptans. Mercaptans also have strong unpleasant aromas that are reminiscent of cabbage, garlic, and skunk. Although sulfur dioxide and sulfides both contain the element sulfur, there is no danger of added SO_2 forming H_2S.

Sulfur dioxide itself is a gas that readily dissolves in water, once dissolved it reacts with the water to form new compounds called **sulfites** as shown in the following reaction:

$$H_2O + SO_2 \leftrightarrow H^+ + HSO_3^- \leftrightarrow 2H^+ + SO_3^=$$

In solution with H_2O (water), SO_2 is called **molecular** SO_2, HSO_3^- is called **bisulfite** and $SO_3^=$ is called **sulfite**. The negative signs (- and =) denote the negative charge of the bisulfite and sulfite ions (molecules with a charge are called ions). The double arrows (↔) of the equation denote that the reaction is at equilibrium. At equilibrium, the rate at which bisulfite ions become sulfite is the same as the rate sulfite ions become bisulfite. The reaction between the different types of sulfite is going both ways at a steady state so the concentration of the sulfite compounds remains constant.

While the concentration of the different forms of sulfites may be steady, it does not mean there are equal amounts of the compounds in solution; the acidity or pH of the water has a huge affect on their concentration. The more acidic or the lower the pH of the water, the more heavily the reaction is weighted to the molecular SO_2 side. The more basic or higher the pH is, the more sulfite that is present as illustrated in Figure I.

How does it differ when SO_2 dissolves in wine? Since wine is more than 80%

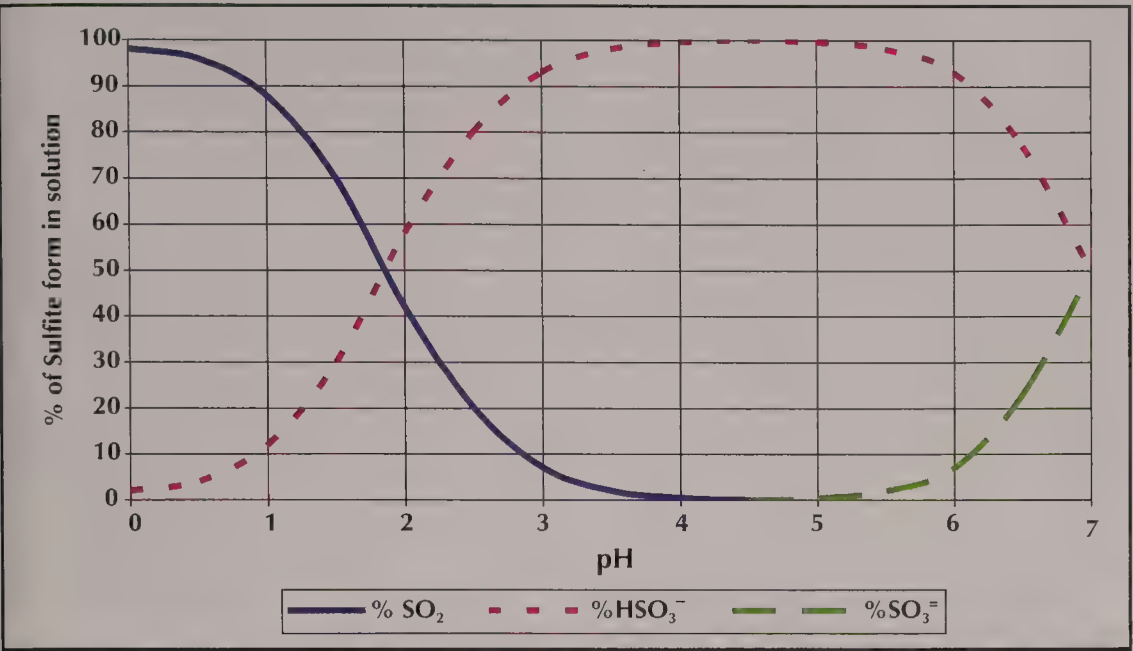

Figure I. The percentage of the different forms of free sulfite over the pH range (0 to 7). Wines usually range from pH 3 to 4, so bisulfite (HSO_3^-) is the dominant form of free sulfite in wine.

Total sulfur dioxide			
Free sulfur dioxide			**Bound sulfur dioxide**
Molecular SO_2	Bisulfite HSO_3^-	Sulfite $SO_3^=$	Sulfites attached to sugars, acetaldehyde and phenolic compounds

Figure II. Different forms of SO_2

water, the reaction, or **disassociation**, of molecular SO_2 into sulfites happens in much the same manner. Since wine is acidic (between 3 and 4 pH), molecular SO_2 and bisulfite make up the vast majority (99.99% at pH 3.4) of the sulfite compounds present in wine.

Sulfites will also react with other chemical constituents found in wine such as sugars, acetaldehyde, and phenolic compounds. When a sulfite reacts with another molecule and becomes part of its structure it no longer takes part in the equilibrium reaction and it is called **bound**. Sulfites that still are part of the equilibrium reaction are called **free**. The combined amounts of free and bound sulfites are called **Total SO_2**.

The more compounds that are available in a wine for sulfites to bind to, the higher the ratio of bound to total sulfites there will be. Therefore, wines that are sweet or have high solids because they have not been settled or filtered will have a lower ratio of free to total SO_2.

When sulfite binds to anthocyanins (the phenolic molecule that gives red wines their color), the anthocyanins go from a colored to a colorless form. Red wines that receive an SO_2 addition will have a slightly less intense color. This effect is particularly noticeable in light colored reds or rosé wines.

Knowing both the amount of free and total sulfites is very important because only the free forms of sulfites are available for providing a preservative role in wine. This is often expressed as ppm free SO_2/ppm total SO_2 to denote which number is free and which is total; these numbers can readily be determined by chemical analysis.

Sulfur dioxide concentration is measured in parts per million (ppm) or as milligrams per liter (mg/L). A wine with 19 ppm free SO_2 and 51 ppm total SO_2 would be noted with 19/51. The amount of bound sulfites would be equal to the total SO_2 minus the free SO_2, (51 – 19 = 32). For dry table wines the level of free sulfur is usually somewhere around 35% to 75% of the level of total SO_2.

Anti-oxidation role of sulfur dioxide

Oxidative reactions can occur in both red and white wines but are particularly noticeable in the latter. Dissolved oxygen in wine can react with phenolic compounds giving the wine a brownish hue. Another product of this oxidation is the compound acetaldehyde, which has a nutty, sherry-like aroma. Sulfur dioxide is used by winemakers to prevent this oxi-

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Table I: Table of molecular SO_2 concentrations over pH

pH	% of Free Sulfur Molecular SO_2	ppm free for 0.8 Molecular	ppm free for 0.5 Molecular
2.90	7.5	11	7
2.95	6.6	12	7
3.00	6.1	13	8
3.05	5.3	15	9
3.10	4.9	16	10
3.15	4.3	19	12
3.20	3.9	21	13
3.25	3.4	23	15
3.30	3.1	26	16
3.35	2.7	29	18
3.40	2.5	32	20
3.45	2.2	37	23
3.50	2.0	40	25
3.55	1.8	46	29
3.60	1.6	50	31
3.65	1.4	57	36
3.70	1.3	63	39
3.75	1.1	72	45
3.80	1.0	79	49
3.85	0.9	91	57
3.90	0.8	99	62
3.95	0.7	114	71
4.00	0.7	125	78

Adapted from: Enology Briefs 1 (#1), Feb/Mar 1982. University of California Cooperative Extension

dation; however it does not act by directly removing oxygen from wines and musts.

Although the sulfite ion ($SO_3^=$) can bind with oxygen, there is very litle sulfite ion present in solution at the pH range found in wine (see Figure I). Rather sulfur dioxide prevents oxidation by binding with the precursors involved in oxidative reactions preventing them from reacting with oxygen or by binding with compounds already oxidized to reverse oxygen's effect. Sulfur dioxide also acts by reducing the activity of the degenerative enzyme tyrosinase (polyphenol oxidase), which is present in juice.

Because of sulfur dioxide's ability to bind with the precursors and the products of oxidation, it can be used as both a preventative and a treatment. For example, an oxidized white wine with a brown tint and a nutty smell can be improved with an addition of sulfur dioxide that bleaches out some of the dark color and binds with the acetaldehyde to reduce the nutty smell.

Antimicrobial role of sulfur dioxide

Sulfur dioxide is a broad-spectrum antimicrobial agent that has an inhibitory affect on a wide variety of microorganisms. The level of sulfur dioxide at which the microbe, either yeast or bacteria, is affected varies widely by species. This variation allows winemakers to use sulfur dioxide to treat microbes in the wine or must selectively and inhibit or kill undesirable microbes without harming the desirable ones.

It has been understood since the early 1900s that only the free forms of sulfur dioxide (and not the bound) have an antimicrobial effect. It was further discovered in the 1960s that molecular SO_2 was several hundred times more effective than bisulfite.

The mechanism for sulfur dioxide's antimicrobial affect works by the sulfur dioxide entering the microbe and disrupting the activity of the enzymes and proteins of the cell. Since only the molecular form of sulfur dioxide can enter through the cell membrane, it is the concentration of molecular sulfur dioxide that controls microbial growth.

Although there is some evidence that a high concentration of acetaldehyde-bound bisulfite inhibits the growth of some species of malolactic bacteria, this effect is eclipsed by the role of molecular SO_2. Since the percentage of free SO_2 that is in the molecular form is dependent on pH, the importance of pH in the effectiveness of sulfur dioxide and the microbial stability of wine in general cannot be overstated.

Figure III: Formula for potassium metabisulfite addition

$$\frac{(\text{gallons of wine}) \text{ x } 3.785) \text{ x } (\text{ppm of addition})}{(1000) \text{ x } (0.576)} = \text{grams of KMB to add}$$

3.785 is the conversion from gallons to liters
1000 converts mg/L (ppm) to g/L
0.576 is the fraction of SO_2 in KMB

This formula can be simplified to:

(gallons of wine) x (ppm of addition) x (0.0066) = grams of KMB to add

Effect of sulfur dioxide on yeast and malolactic bacteria

Sulfur dioxide has some degree of inhibitory affect on all yeast; however, the *Saccharomyces* yeast strains that are used by winemakers for alcoholic fermentation are much more resistant to it than "wild" yeasts are. Wild yeast is the term used for a number of non-*Saccharomyces* species of ambient yeast that are present on grapes and in the winery cellar. Wild yeast are sensitive to both sulfur dioxide and alcohol.

Wild yeast can begin a spontaneous fermentation in juice but soon are killed by the alcohol that they produce. At this point, the *Saccharomyces* that is present, either indigenous to the grapes or added by the winemaker, which is more resistant to alcohol takes over to complete the fermentation.

Wild yeast can sometimes be the source of off-flavors therefore most winemakers elect to control them with a small dose of sulfur dioxide and then inoculate the must with a commercial wine strain of *Saccharomyces*. A molecular SO_2 level of 0.4 ppm (equivalent to a free SO_2 level of 20 ppm at 3.50 pH) will kill wild yeast without adversely affecting *Saccharomyces*.

The inhibitory effect of sulfur dioxide on malolactic fermentation is much greater than it is for the alcoholic fermentation that is performed by *Saccharomyces* yeast. Malolactic fermentation (often abbreviated to ML fermentation or MLF), is the secondary fermentation by bacteria that converts malic acid found in grape juice to lactic acid, which is less acidic.

There are several species of bacteria that are capable of MLF in wine, some more desirable than others. *Oenococcus oeni*, the most commonly used species of malolactic bacteria, is very sensitive to sulfur dioxide and has difficulty growing at levels above 25 ppm total SO_2.

The sensitivity of malolactic bacteria to sulfur dioxide can be used by winemakers to influence the flavor of a wine. MLF has several effects on the flavor of a wine, it lowers the acidity and it can produce a "buttery" aroma, particularly in white wines. In addition to these sensory qualities, it makes a wine more microbiologically stable.

If the MLF is completed in the cellar prior to bottling, you do not have to worry about it occurring after bottling and spoiling the wine. If you wish to make a more tart, fruit forward wine such as a Riesling or Sauvignon Blanc, MLF would not be as appropriate and MLF can be prevented by an early dose of sulfur dioxide.

In a wine that will be aged for a longer period in barrels, or if you desire a softer less acidic character, MLF is more appropriate. Chardonnay and red wines often are put through MLF either prior to or during ageing.

Effect of sulfur dioxide on wine spoilage microorganisms

Although there are no human pathogens that can grow in wine, there are a number of spoilage bacteria and yeast that can adversely affect a wine's flavor. The most common of these are *Acetobacter, Lactobacillus, Pediococcus,* and *Brettanomyces.* All of these are sensitive to some degree to sulfur dioxide but the best results come from a combination of sulfur dioxide and good cellar practices.

Acetobacter is also known as acetic acid or vinegar bacteria. As the name implies, it can grow in wine and produce vinegar (acetic acid). *Acetobacter* can only develop in the presence of oxygen and often becomes a problem in wines that are being aged in un-topped containers. It can be controlled with a combination of sulfur dioxide and keeping tanks and barrels topped and full after fermentation.

Lactobacillus and *Pediococcus* are both forms of undesirable malolactic bacteria. *Lactobacillus* typically grows in high pH red wines that have a stuck fermentation. It grows on the sugar present from the incomplete alcoholic fermentation and produces large quantities of acetic acid. Because it usually grows in high pH musts (greater than 3.75), sulfur dioxide is less effective. The best treatment is prevention, adding acid to a high pH must, and maintaining a healthy fermentation that completes to dryness.

If *Lactobacillus* has already become established, lysozyme (an antimicrobial enzyme that is effective at high pH), can be added to control growth. *Pediococcus* produces an off-aroma that is described as "vegetal" or "dirty socks" and often comes from cooperage that has not been kept clean. Like all spoilage microbes, it can be prevented by a combination of proper sanitation of winery equipment and cooperage, adding pure inoculums of yeast and malolactic bacteria for fermentation, and the addition of sulfur dioxide.

Brettanomyces is yeast that can grow in wine without the presence of oxygen or sugar. It usually shows up in high pH red wines while they are being aged and it produces an aroma that is described as "barnyard" or "horse sweat." *Brettanomyces* grows very slowly and some winemakers feel that a small amount of its aroma can add complexity to a wine. However, too much of this character can be seen as a flaw and most winemakers avoid it.

Sulfur dioxide usually does not kill *Brettanomyces* but prevents it from growing. As a wine ages, the level of free SO_2 diminishes, and a wine that has been adjusted with sulfur dioxide prior to being placed in barrels may require supplementary additions as it is being aged to prevent Brettanomyces from developing.

When to add sulfur dioxide

The flavor of white wines always benefits from the preservative nature of sulfur dioxide. The timing of the addition, whether it is added before or after alcoholic fermentation, has a huge affect on a wine's ultimate character. If sulfur dioxide is added prior to alcoholic fermentation, the enzyme polyphenol oxidase is inhibited and less oxidative browning of the juice occurs. This helps to preserve the fruity and floral aromas found in the juice.

The presence of sulfur dioxide will also inhibit malolactic bacteria and help to prevent malolactic fermentation (MLF) from occurring, leaving more of the natural acidity in the final wine. This method is preferred for fruit-forward wines with crisp acidity that will not receive a great deal of barrel ageing.

If sulfur dioxide is not added prior to alcoholic fermentation, more oxidative browning will occur and, within a few days of pressing, the juice will take on a

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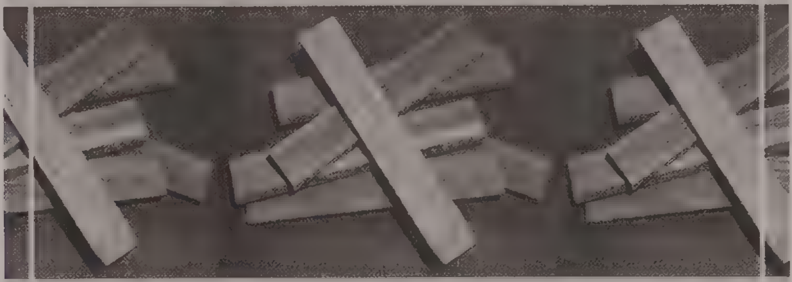

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Figure IV: Example of a sulfur dioxide addition calculation

A Zinfandel has just finished aging in a barrel for one year and needs its sulfur dioxide level adjusted before it is ready to be bottled. It is dry and has completed malolactic fermentation and has very good clarity from setting during ageing. The analysis before the addition is pH 3.45, and the sulfur dioxide is 16/35 and there are 60 gallons of wine.

Q: How many grams of KMB should be added to protect the wine after it has been bottled?

A: Since the wine has completed both the alcoholic and malolactic fermentations a molecular SO_2 level of 0.5 ppm should be adequate to preserve the wine. By looking at the chart in figure 3 you can see the free SO_2 must be 23 ppm to have a molecular SO_2 level of 0.5ppm. With the free SO_2 currently at 16 ppm it must increase by 7 ppm to attain the desired amount.

23 ppm – 16 ppm = 7 ppm

Since only a portion of the KMB that is added will be free SO_2 it is necessary to estimate what the percentage that will become free SO_2. Since the wine is clean and dry, we can assume that the ratio will be on the high side of the 35 to 75% range and for this example we will use 70%.

7 ppm ÷ 0.7 = 10 ppm

To determine the amount of KMB when adding 10 ppm:

(10 ppm) x (6,000 gallons) x (0.0066) = 3.96 grams of KMB

If you wish to convert this to pounds (one pound is approximately 454 grams):

396 grams ÷ 454 grams per pound = .872 pounds

For an aqueous solution of sulfur dioxide:

$$\text{volume of aqueous } SO_2 \text{ in mL} = \frac{\text{PPM addition}}{2.6} \times \frac{\text{gallons wine}}{\% \ SO_2 \text{ solution}}$$

For the wine used in the KMB example using 5% aqueous SO_2.

10/2.6 x 6,000/5 = 4,615 mL

There is a certain amount of guesswork in estimating just how much of a sulfur dioxide addition will be available as free SO_2 and it is always best to be conservative—you can always add more, but you cannot add less.

brownish hue. While this dark color may cause alarm, you should not be overly concerned because after fermentation the dark, oxidized phenols will settle out, leaving a wine that is much brighter and more appropriate in color for a white wine.

Since the phenols that can oxidize have been removed from settling and racking, there is less of a potential for the wine to oxidize after fermentation and the wine will be more color-stable and better able to age. This method of pre-fermentation oxidation does diminish some of the fruity and floral aromas in the final wine.

The absence of sulfur dioxide allows MLF to commence after primary fermentation is complete, lowering the acidity of the wine, and making it more microbiologically stable. After MLF is finished, sulfur dioxide should be added to protect the wine. If MLF is not desired, the wine should be adjusted with sulfur dioxide after primary fermentation is complete.

For red wines, it is a good idea to add a small amount (about 30 to 40 ppm), of sulfur dioxide immediately after the grapes have been crushed. While not absolutely necessary, this will discourage the growth of spoilage organisms such as Lactobacillus and allow the yeast to get a good start on the fermentation without competition from other microbes.

By the end of primary fermentation, the majority of SO_2 will be bound up and there will not be so much residual SO_2 that the growth of malolactic bacteria is inhibited. After MLF is complete, sulfur dioxide should be added to protect the wine during ageing.

For both red and white wines, as they age, free sulfites are bound by other compounds or are oxidized to sulfate anion resulting in a gradual lowering of the effective amount of sulfur dioxide in wine. For this reason, it is always a good idea to monitor the SO_2 level during ageing and to add more as needed.

How much sulfur dioxide to add?

Since it is the molecular form (SO_2) of sulfur dioxide that has the most potent antimicrobial effect, and the percentage of sulfite that is in the molecular form is directly dependent on the pH, one must always consider both the pH and the free SO_2 when deciding how much sulfur dioxide to add to a wine.

By knowing the pH, you can determine the percentage of free sulfur that is in the molecular (SO_2) form by using Table I.

For white wines, a level of 0.8 ppm molecular SO_2 will slow down the growth of yeast and will prevent the growth of most other microbes. This level of sulfur dioxide will bind up most of the acetaldehyde in a wine and reduce any oxidation aroma considerably. Therefore, 0.8 ppm is a good target level for molecular SO_2immediately prior to bottling and will provide the maximum protection for the finished wine.

However, sensitive tasters will be able to detect a slight burnt match aroma at 0.8 ppm SO_2. This is usually not a problem however because few consumers will be able to detect it. Additionally if the wine is bottle-aged for a few months before consumption, the SO_2 will decrease as more sulfites react with other chemical constituents in the wine and become bound. Thus, a wine bottled at 0.8 ppm will decrease to a lower level fairly quickly and there would be no detectable sulfur dioxide aroma.

Winemakers who seal their wine with screw caps know that the sulfur levels diminish more slowly after bottling than wines sealed with corks. In this case, 0.7 ppm would be a better target for molecular SO_2 at bottling. During storage, after all fermentations have completed, white wines should be adjusted to between 0.5 and 0.8 ppm molecular. If the wine is sweet or if you wish to prevent MLF, the wine should be kept at the high side of this range.

Total SO_2 should be kept below 110 ppm for table wines because, at higher levels, the wine can acquire off-flavors. For dessert and fortified wines, that are very sweet, it may be necessary to exceed this limit to obtain adequate free SO_2.

For red wines, a level of 0.5 ppm molecular SO_2 at bottling is an appropriate target. You do not need to keep the molecular SO_2 as high on red wines as you do white wines for several reasons:

First, in most cases, MLF is complete in reds so there is no need to try to discourage it.

Second, red wines are less sensitive to oxidation and their flavor is less dependent on fresh fruity aromas so sulfur dioxide's preservative effects are not as critical.

Third, red wines usually have a higher pH than whites and often it is not possible to adjust the sulfur dioxide to a level that reached 0.8 ppm molecular SO_2 without having too much total SO_2.

By knowing the pH of the wine and using the chart in Table I, it is easy to see what level of free SO_2 is necessary to obtain a given level of molecular SO_2. However, it is more difficult to determine precisely what the ratio of free to bound sulfur dioxide level will be.

When adding sulfur dioxide, the amount in ppm that you add should change the total SO_2 by the same ppm. However, because some of the sulfur dioxide that you add will become bound, the level of free SO_2 will change by a fraction of the amount that is added.

Free SO_2 in wine ranges from about 35% to 75% of the total SO_2 depending on the amount of compounds that are available in the wine to which the sulfite molecules can bind. To approximate what the ratio will be, use the 35% level for wines that are turbid or sweet and the 75% level for clean, dry wines.

The higher the level of total SO_2 in the wine, the higher the ratio will be, because there are fewer unbound compounds available for reacting with additional sulfur dioxide as it is added. Sulfur dioxide is also more affective if it is added less often and in greater quantities because it will be more of a shock to the microbes. For example, one addition of 30 ppm is more effective at killing microbes than two additions of 15 ppm spread several weeks apart.

Forms of sulfur dioxide to add

Sulfur dioxide is available in its pure form as a compressed gas that can be made into an aqueous solution for wine additions. Most wineries use a stable, powdered form of sulfur dioxide called potassium metabisulfite. Potassium metabisulfite has the molecular formula of $K_2S_2O_5$ and is 57.6% available SO_2 by weight. Potassium metabisulfite is often abbreviated as KMB or sometimes PMBS or KMBS (K is the chemical symbol for potassium). It should be noted that KMB, PMBS and KMBS are abbreviations and not chemical formulas.

The formula and calculations for determining how many grams of KMB you need to add for a given rise in ppm of SO_2 is shown in Figures III and IV. For convenience, pre-weighed effervescent tablets are also available for additions to barrels.

Some wineries prefer to use a premixed aqueous solution of sulfur dioxide rather than KMB. The liquid is typically 5% to 10% SO_2 by weight and it can be purchased or made up at the winery by dissolving SO_2 gas or KMB into distilled water. The liquid can be directly added to wine without mixing and the proper amount is measured volumetrically instead of weighed on a scale. There is an example of the calculation for a sulfur dioxide addition to wine in Figure IV.

Sulfur dioxide is also available in another powdered form, sodium metabisulfite. Solutions of sodium metabisulfite in water make an excellent sanitizing agent for winemaking equipment but it should **not** be used for making additions to wine. Although it is not toxic, the sodium in sodium metabisulfite is not healthful and it is best left out of wine.

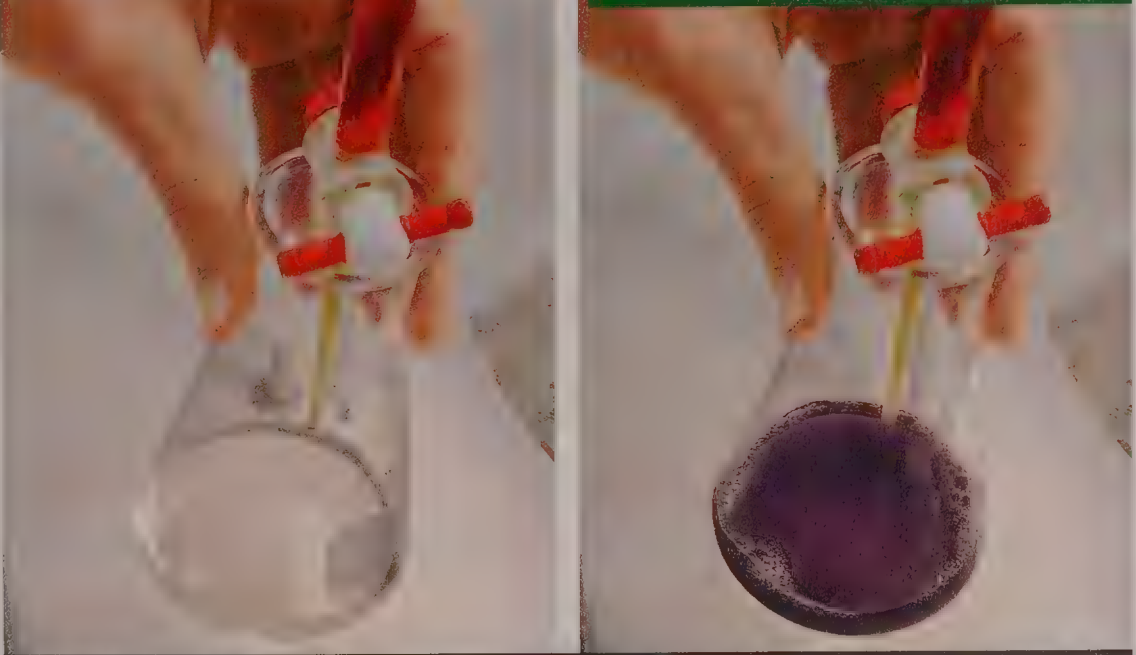

Figure V-a and V-b: At the endpoint of the Ripper titration, the indicator goes from colorless to blue. This is easy to see in white wines as demonstrated in the before, V-a (left) and after, V-b (right).

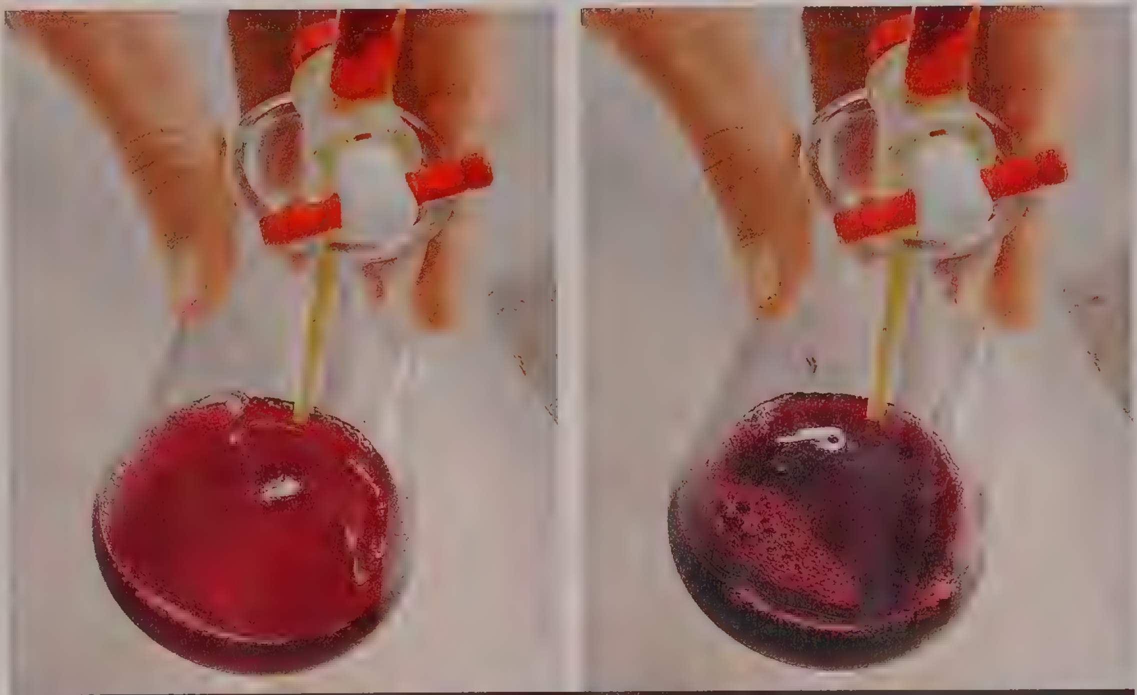

Figure V-c and V-d: For red wines, the blue color of the endpoint is obscured by the red color of the wine and is less obvious, as shown in the before, V-c (left) and after, V-d (right).

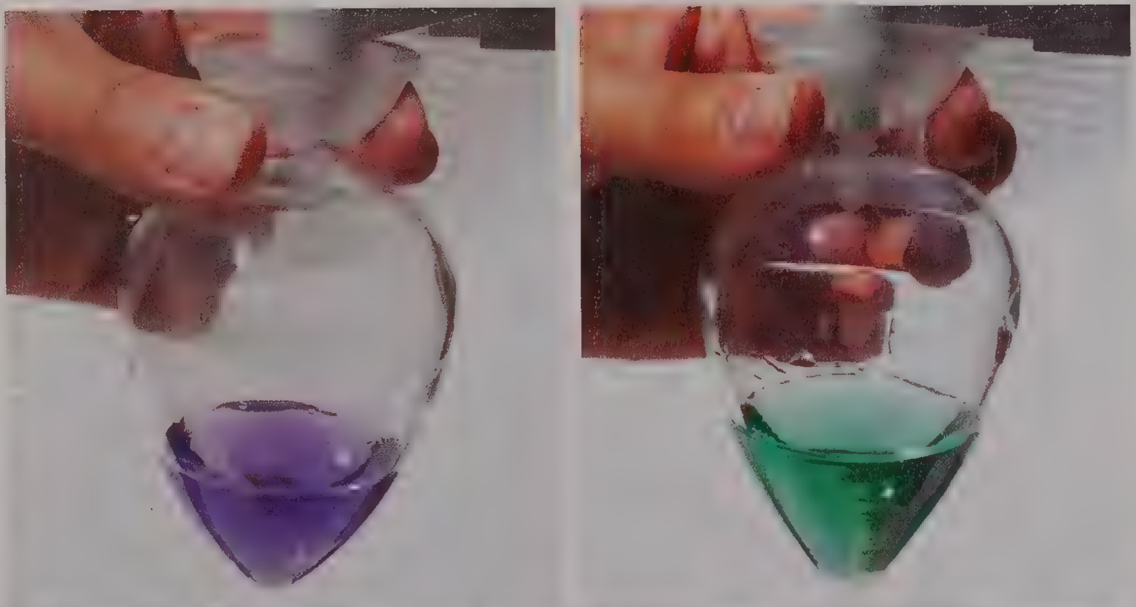

Figure V-e and V-f: In the aeration/oxidation method the sulfur dioxide is aspirated out of the wine sample before titration so the endpoint is not affected by the color of the wine. Before and after the endpoint are shown in figures V-e (left) and V-f (right).

Measuring sulfur dioxide

The exact amount of both free and total sulfur dioxide in a wine or must can only be determined by chemical analysis. Two primary methods that are used are known as the Ripper method and the Aeration-Oxidation method. Both methods have limitations and require an investment in laboratory equipment and chemicals and a degree of expertise in laboratory practices.

The Ripper method works much better with white wines than it does for red wines. At the endpoint of the ripper reaction, the indicator goes from colorless to indigo blue as seen in a white wine in Figures V-a and V-b. The blue color of the endpoint in the reaction is much more difficult to determine in deeply colored red wines as seen in Figures V-c and V-d.

In the Aeration-Oxidation method the endpoint of the titration is not affected by a wine's color and goes from purple to light green, as seen in Figures V-e and V-f. For this reason, the Aeration-Oxidation method is preferred for red wines.

If the Ripper method is the only method available, red wines can be diluted 50% before analysis to lower the intensity of the color. Just be sure to double the results to make up for the dilution. Additionally for red wines, the free sulfur dioxide by ripper usually runs slightly higher than the actual amount. After adding sulfur dioxide, wait 24 hours before performing analysis to allow the free SO_2 to stabilize.

Working with sulfur dioxide

Working with the powdered forms of sulfur dioxide is not a dangerous activity but there are certain precautions that should be taken. Sulfur dioxide at the levels that are found in wine are not high enough to cause for concern however winemakers are exposed to greater levels when they are working with sulfur dioxide solutions.

Fumes of sulfur dioxide gas can irritate the throat and eyes so it is always best to work in a well-ventilated place and to use a respirator when mixing sulfur dioxide solutions. When adding KMB, first mix the powder in a small amount of cold water (about 50 g per Liter, [7 ounce per gal]), then after it has dissolved, mix the solution into the wine.

Additionally, under highly acidic conditions, the concentration of the more volatile molecular form SO_2 is higher, so you should never mix both acid and sulfites together in the same container when they are being added to wine. A better method is when adding both acid and KMB is to first mix and add the acid to the wine and then add sulfur dioxide. A very small portion of the population can have an intense asthmatic reaction when exposed to the fumes of sulfur dioxide, so extra care should be taken for anyone with a history of asthma when working with sulfites.

Summary

Sulfur dioxide is one of the most effective tools that a winemaker has to protect a wine's quality and influence what it will taste like. Deciding when and how much sulfur dioxide to add depends on what stage of winemaking the wine is in and what you are trying to accomplish with the addition. Furthermore, to determine the proper quantity of potassium metabisulfite to use for the addition, you need to know the pH and free and total sulfur of the wine.

With any technique used by a winemaker, it is important to have knowledge of the science behind the skill. By understanding the chemistry of sulfur dioxide and what reactions that occur when it is added to wine, you can make decisions that are based on your goals for what you want the wine to ultimately taste like rather than just following a "one size fits all" recipe. PWV

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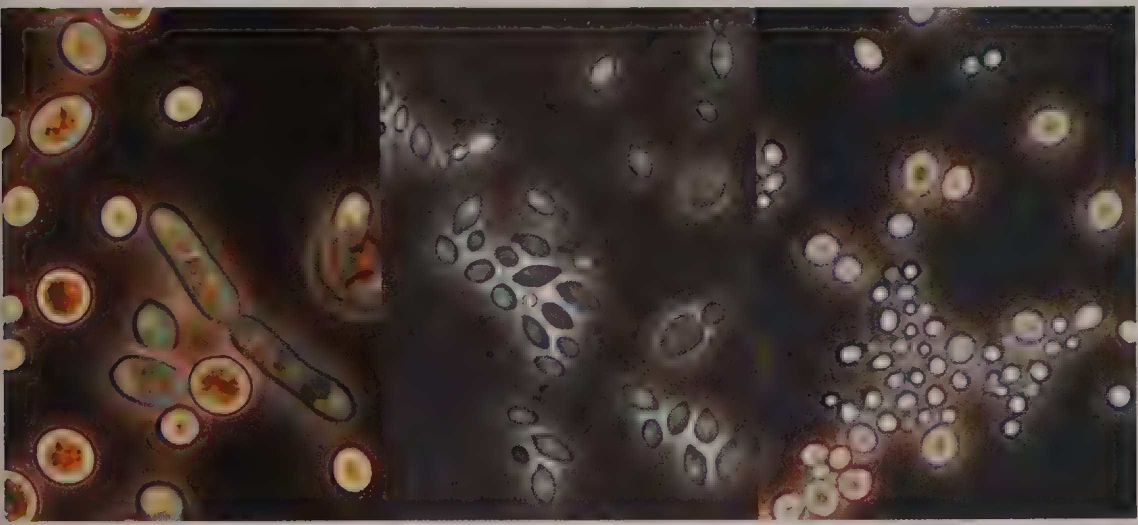


PHOTOS: LEFT + MIDDLE, LISA VAN DE WATER; RIGHT, NICOLA WILTON

Left: Non-*Saccharomyces* yeast in stuck wine; Center: *Kloeckera/Hanseniaspora* with two *Saccharomyces* cells; Right: Non-*Saccharomyces* (small) and *Saccharomyces* in stuck Chardonnay.

microbes

monitoring during fermentation

BY Lisa Van de Water

Winemaking involves encouraging cooperation with microbes we want, and discouraging participation from microbes we do not want at all, or do not want at the time (for example, *Saccharomyces* in a bottled sweet wine). Levels of concern vary from wine to wine and from winery to winery, but there are some guidelines to start from.

Monitoring microbes effectively and efficiently during the winemaking process requires coordinating several different techniques. Examination with a phase-contrast microscope, culturing on various media, genetic testing using polymerase chain reaction (PCR), usually quantitative PCR-based tests including Scorpions® and others, chemical tests, sensory cues, and others all have a place in today's wine microbe detection protocol.

Especially during the harvest season, access to a good phase-contrast microscope is an extremely valuable tool, because samples can be examined directly without staining. Many problems can be detected in a few minutes, so winemakers can respond immediately. A brightfield microscope can be used to examine yeasts after staining, but not to detect wine bacteria reliably. Despite the cost (several thousand dollars), access to a high-level phase-contrast microscope is recommended for all wineries (groups of wineries could purchase one together).

Please see "Spoilage microbe populations fluctuations during winemaking—causes, effects, solutions" by Robert Tracy, May/June 2009 PWV Journal.

This text edited from first publication: Jan./Feb. 2010.

Even with such a microscope, the lower limit of detection by direct microscopic exam is around 2,000 cells/ml. Fortunately for the wine microbiologist, however, microbes involved in spoiling fermentations are present at higher levels — often much higher.

BEFORE FERMENTATION

Microbes to watch out for: Non-*Saccharomyces* yeast species, lactic acid bacteria, acetic acid bacteria

Danger signals: Mold and/or rot, signs of spontaneous fermentation, acetic acid (vinegar) or ethyl acetate odor

Monitoring methods: Sensory cues, microscopic exam, PCR-based genetic testing

Mold/Rot

If grapes arrive with mold and/or rot, take photos and save an uncrushed sample to freeze immediately as a record of the grapes' condition. Test laccase (responsible for the browning of white wines and discoloration of red wines) and volatile acidity (VA), and consider rejecting fruit if VA is more than 0.1 g/L in juice. Note that crushing of grapes may be considered legal acceptance of the grapes "as is."

After grapes that were not completely healthy are processed, take a sample from the tank and examine it under the phase-contrast microscope for bacteria and yeast. Fortunately, molds are very intolerant to alcohol, so they die as soon as fermentation begins. It is normal to see a few yeasts, but no more than about 5 per 40x field. There should be no bacteria, or at most one rod-shaped bacterium in several 40x fields. Even one *Acetobacter/ Gluconobacter* cell seen under the microscope is too many!

If any *Lactobacilli* are seen, add lysozyme to the must immediately, or continue to monitor the batch microscopically once per day, to see if the population increases. Also monitor growth of non-*Saccharomyces* yeasts, as discussed below.

Non-*Saccharomyces* yeasts from grapes and in wineries

There are many yeasts on grapes, though very few are *Saccharomyces*. This surprises most winemakers who assume that *Saccharomyces* is the dominant genus in vineyards, despite extensive scientific studies to the contrary. *Kloeckera apiculata* (teleomorph *Hanseniaspora uvarum*) is a major species, and there are about 100 other species in vineyards and wineries.

Some species are very sensitive to alcohol, or are so aerobic (requiring air) that they cannot grow during fermentation, but they may grow before or during the start of fermentation. Other species may survive until middle to late in the fermentation process.

Many yeast species—often originally from grapes—take up residence in winery cellars, and begin to colonize new grapes as soon as they arrive at the winery, even in the crusher/destemmer. When juice/must is not inoculated with cultured yeast, or if the cultured yeast has been mishandled, non-*Saccharomyces* yeasts may grow before or at the start of fermentation, sometimes at the expense of a healthy *Saccharomyces* population that can ferment to dryness.

If odors of ethyl acetate (airplane glue/nail polish remover), amyl acetate (banana skin), or other off-odors appear in juice or must, examine immediately under a phase-contrast microscope for yeasts that are not the round/ovoid shape of *Saccharomyces*. Non-*Saccharomyces* yeasts are sometimes round, but more often have other appearances: apiculate (pointy), elongated, or bizarrely shaped, and they may be unusually small or large.

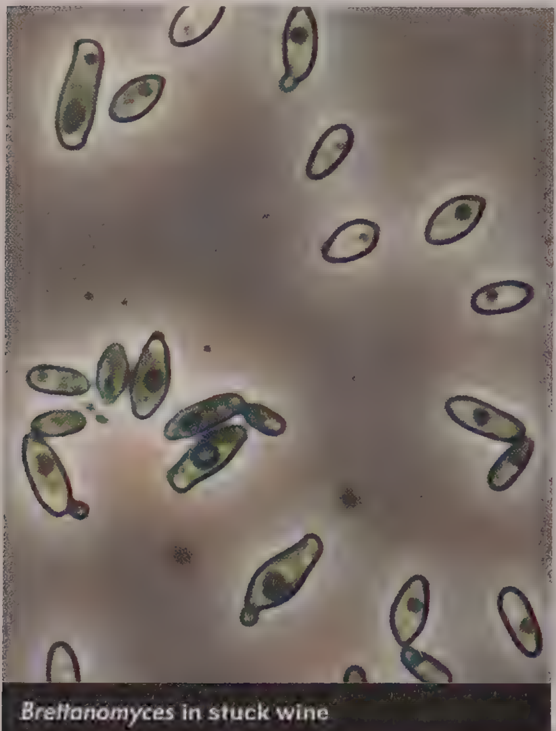
Kloeckera/Hanseniaspora is especially problematic for several reasons. Their SO_2 sensitivity is comparable to that of *Saccharomyces*, and they are naturally cold-tolerant so they grow readily in cool must (10–15° C/50–60° F). They can survive much farther into the fermentation than most other non-*Saccharomyces* species, occasionally all the way to the end.

Sometimes a thick, slimy scum forms, or stringy clumps that fall out later as fluffy lees. *Kloeckera* can make prodigious amounts of ethyl acetate and amyl acetate, and is quite efficient in quickly depleting nutrients, particularly thiamine and other vitamins. Microscopically, it often resembles bowling pins or thin lemons with a knob on each end.

Kloeckera is easy to culture because it is cycloheximide-resistant and grows in one to two days, unlike *Brettanomyces* (three to seven days), which *Kloeckera* resembles. There are also PCR primers for *Kloeckera*, and for *Pichia membranifaciens*, which could be used at this stage, although there are no primers for most other non-*Saccharomyces* vineyard species, so those will not be detected by PCR-based methods.

However, the question is not whether there are *Kloeckera* and other vineyard yeasts in the must (there are), but how to monitor their population, and how to handle the must so that they do not grow too extensively. For this purpose, following the must microscopically, estimating percentages of non-*Saccharomyces* species, is more useful than any other test methods. Before fermentation begins, if more than 5 non-*Saccharomyces* yeasts are seen in a 40x field, a problem is likely to be developing.

Examine some musts microscopically every day, especially any must over 10° C/50° F that are soaking on skins or warming up from soaking/settling, or musts that start fermenting spontaneously. Also examine microscopically if acetate or amyl acetate odor is noted.



Brettanomyces in stuck wine

PHOTO BY LISA VAN DE WATER

YEAST FERMENTATION

Microbes to watch out for: Non-*Saccharomyces* yeasts, lactic acid bacteria
Danger signals: Spontaneous fermentation, ethyl acetate or other off-odor, sluggish fermentation, spontaneous malolactic fermentation (MLF), low viability of yeast inoculum, VA increase
Monitoring methods: Microscopic exam, sensory cues, chemical tests, PCR-based genetic testing

Saccharomyces

When preparing dry yeast, check viability with methylene blue stain to make sure that enough survived transport, storage, and rehydration in the winery. Approximately half of the rehydrated yeasts should be viable, as evidenced by lack of blue staining. Counting yeasts with a hemocytometer also helps to monitor the population to be sure that it reaches 10^8 cells/ml, or a stuck ferment is likely.

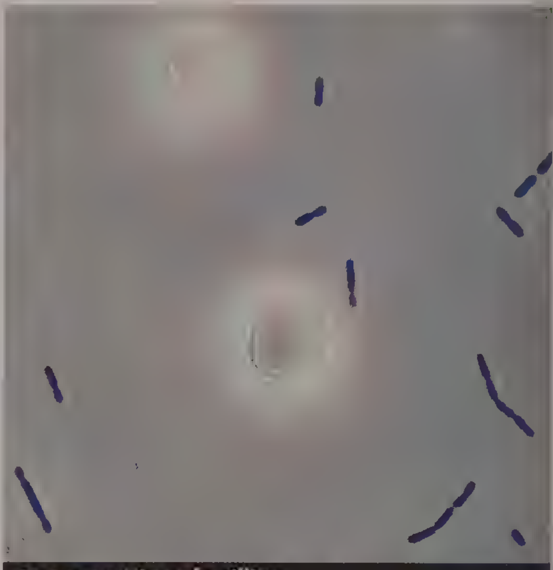
Non-*Saccharomyces*

Some winemakers encourage the growth of some non-*Saccharomyces* vineyard species to contribute to aromatics, complexity and mouthfeel before *Saccharomyces*, which must become dominant for the fermentation to go to completion, takes over. Potential problems with this approach include depletion of vitamins and nutrients (natural and added) that *Saccharomyces* will need, production of off-characters such as H_2S, ethyl acetate and acetic acid, and possibly inhibition of *Saccharomyces*.

It is difficult to over-emphasize the potential for problems that can result from too much growth by non-*Saccharomyces* yeasts.

It is very important for winemakers encouraging participation by vineyard yeast species to guard vigilantly against their extensive growth. Many stuck fermentations have their origins early on, as growth of competing species did not allow *Saccharomyces* to achieve a large population of healthy yeasts. The winemaker may look for a cause late in fermentation, but what happens at the beginning can set the process in motion.

Spontaneous fermentations, whether intentional or not, should be checked microscopically until *Saccharomyces* becomes the dominant yeast. If *Saccharomyces* does not take over within the first day of fermentation, a large inoculum of a vigorous active-dry yeast may be required (even if no yeast addition was originally planned). If non-*Saccharomyces* yeasts reach 10^6/ml or more, the population may need to be reduced by centrifuging or filtering, before inoculating with properly rehydrated dry yeast.



Yeast and *Lactobacilli* in stuck wine

PHOTO BY PATRICIA ROCA

Nutrient additions to uninoculated fermentations should be delayed until *Saccharomyces* is recognized under the microscope, or until a clean-smelling fermentation is under way. Fermentations with more than around 10% non-*Saccharomyces* yeasts should be considered at high risk and carefully watched for signs of sluggishness.

Lactic acid bacteria

Spontaneous growth of lactic acid bacteria during fermentation can sometimes be a positive occurrence, if the bacterium is *Oenococcus oeni*, MLF is desired, and no trouble in finishing the yeast fermentation is expected.

For many years, beginning in the 1970s, red musts were often inoculated with *Oenococcus* once yeast fermentation was fully active, with very good success in completing both fermentations together (correctly called "co-fermentation" not "co-inoculation," because the yeast and bacteria are not added at the same time).

But if certain hetero-fermentative *Lactobacillus* species (called "ferocious" by the late Dr. Ralph Kunkee) grow when there is fermentable sugar, the result can be a disaster, traditionally called "piqure lactique." This is more common in musts with higher pH (above 3.6). Besides converting malic to lactic acid, they metabolize glucose and fructose to acetic acid, raising VA to 1 g/L or higher.

New research from Dr. Linda Bisson's lab at U.C. Davis has shown that some common wine spoilage bacteria and possibly other stresses can lead to a stress-induced "prion state." *Saccharomyces* harboring this prion have a reduced fermentative capacity and are less dominant over microbial competition, yet remain more viable over time.

Acetic acid, along with toxins that at least some species produce, is inhibitory to *Saccharomyces*, and the fermentation may stick. While *Oenococcus* is hetero-fermentative and thus theoretically could cause this type of spoilage, in fact *Lactobacillus* is overwhelmingly the bacterium responsible.

Lactobacillus spoilage may go undetected until too late. Because *Lactobacilli* do not make ethyl acetate, there is no telltale odor, and sugar can mask the acetic "bite" at the finish. Culturing is not recommended because wine *Lactobacilli* tend to grow very poorly in culture. PCR-based tests detect certain lactic acid bacteria, but do not detect all wine *Lactobacilli* and thus miss incidences of spoilage which could have been observed microscopically.

Microscopic examination easily determines whether *Lactobacilli* of any species are growing. If there are enough cells to cause a problem, there will be a large enough population to see. Chemical tests can alert the winemaker to check for *Lactobacillus* spoilage: pH rise, drop in malic or rise in lactic acid, and rise in VA/acetic acid.

STUCK WINES

Microbes to watch out for: *Lactobacilli, Pediococcus, Acetobacter, Brettanomyces*, surface film yeast
Danger signals: CO_2 production, spontaneous MLF, VA rise, film on surface
Monitoring methods: Visual, microscopic exam, PCR, culturing, chemical tests

Stuck wines are extremely susceptible to spoilage. The sugar offers a substrate for a number of microbes, and any efforts at encouraging completion of fermentation, including warming, aerating, and nutrient addition, will also encourage spoilage microbes.

To determine whether non-*Saccharomyces* yeasts helped cause a stuck ferment, take an unstirred sample and then a stirred one.

In the settled lees, there may be large numbers of odd-shaped yeasts, culprits that have died after causing, or contributing to causing, the stuck fermentation.

Summary of Wine Microbes — How Many are TOO Many?

IN FERMENTATION	
Kloeckera	Juice: more than 5 per 40x field During fermentation: more than 10% of yeast population (*Saccharomyces* should take over) Stringy clumps are not desirable
Other vineyard yeast	More than 10% of yeast population
Schiz. japonicus (8 spores)	Any
Saccharomyces	Should have more than 2 million/ml at inoculation; more than 100+ million/ml during ferment Viability: should be 50% at rehydration; more than 90% at start of ferment; more than 25%+ near end of ferment
Dekkera/Brettanomyces	Any (determine by culturing or PCR)
Surface film yeast	1 per 40x field
Oenococcus	At least 1 million/ml if MLF desired Any observed under microscope if MLF not desired
Pediococcus	Any observed under microscope
Lactobacillus	2 per 40x field if population increasing
Acetobacter	Enough to see under microscope
IN ML STARTERS	
Saccharomyces	Should be fermenting with selected, ML-friendly yeast to acclimate bacteria to alcohol
Dekkera/Brettanomyces	Any (determine by culture or PCR)
Surface film yeast	Any observed under microscope
Pediococcus	Any observed under microscope
Lactobacillus	2% to 5% if population is rising and starter is for wine with residual sugar, 10% to 20% if starter is for dry wine
Acetobacter	Any observed under microscope (scan very thoroughly—they may all be in a large clump, not scattered, or in little clumps that are hard to recognize); test with PCR if not sure

Lactic acid bacteria

Stuck and sluggish wines are often attacked by *Lactobacilli* (as described above). Any CO_2 production in a stuck or sluggish wine should be viewed with suspicion, for it may be caused by *Lactobacilli* (or *Brettanomyces*) rather than *Saccharomyces* resuming fermentation.

Monitor stuck wines every day or two under the microscope while reinoculating with fresh yeast. In wines above 3.6 pH, it may be advisable to reduce pH or add lysozyme prophylactically, to reduce the chance of *Lactobacillus* spoilage.

Brettanomyces/Dekkera

Fortunately, very few musts become contaminated with *Brettanomyces*, and anyway, they tend to grow slowly compared to *Saccharomyces*. However, stuck wines may become infected, especially if they are moved to wood to complete the fermentation. To detect *Brettanomyces* as soon as possible, culture any stuck red wine once per week, or check periodically by PCR.

Production of 4-ethyl-phenol/4-ethyl-guaiacol (4-EP/ 4-EG) lags behind cell growth by up to one month. This is not the best way to discover a new "Brett" infection in a stuck wine. Certainly, if apiculate yeasts are seen microscopically in a stuck wine, it should be tested for *Brettanomyces* immediately, but by that time the population may be already quite high.

Film on surface

Both *Acetobacter* and surface film yeasts (mainly *Candida* and *Pichia*) enjoy conditions that prevail in a stuck wine during reinoculation: warm, low SO_2, not topped. Any film should be examined microscopically. To prevent film, the container should be brought as full as possible, and CO_2 or other inert gas used liberally. *Acetobacter* can also be discouraged by lowering pH, but yeasts are not as sensitive to low pH as are bacteria.

Dry wines undergoing extended maceration are also at risk from *Acetobacter* spoilage, because there is not enough CO_2 produced to prevent their growth. Such wines must be gassed regularly with CO_2.

ML FERMENTATION

Microbes to watch out for: *Acetobacter, Lactobacillus, Pediococcus, Brettanomyces, Oenococcus*, surface film yeasts
Danger signals: VA rise above 0.15 g/L (0.015 g/100ml); the amount produced by *Oenococcus* from citric acid in stuck wine, film on surface, off-odors or flavors

Monitoring methods: Sensory cues, microscopic exam, PCR

Most commercial freeze-dried cultures are produced following extensive quality assurance procedures to be sure they are free from contaminants. Once they are put into must or wine, however, the resident bacterial flora may grow along with, or instead of, the inoculated strain(s).

Check wines during MLF for spoilage bacteria, at least microscopically and also by PCR methods, if possible. This is especially important if the culture is propagated before adding it, allowing unwanted lactic acid bacteria more opportunity to grow as well (or instead), and also giving aerobic yeasts and acetic bacteria a chance to spoil the starter. *Acetobacter* growth is particularly unfortunate in ML starters and during MLF. Microscopically, it is sometimes confused with *Oenococcus*.

If *Lactobacillus* and/or *Pediococcus* are a significant part of the wine's bacterial flora, it may be best to kill them with lysozyme and reinoculate with a resistant *Oenococcus* strain. However, if the wine is dry, the winemaker may decide to accept the presence of some *Lactobacilli* during MLF, despite a tendency to introduce "pickle" or "sauerkraut" aromas.

Pediococci can make lactic acid but not acetic acid from sugar. They are considered undesirable mainly because some can continue growing after wine is dry and has finished MLF, and because of biogenic amine production, though off-flavors ("dirty socks") have been ascribed to some strains.

In cellars heavily infected with *Brettanomyces*, culturing once each week or testing with PCR during MLF is recommended. PWV

Lisa Van de Water, known as "the bad wine lady," can be reached at www.vinotecnapa.com or badwinelady@aol.com.

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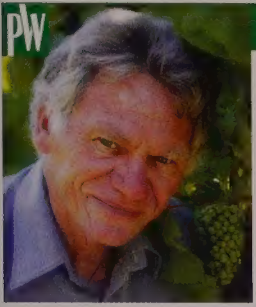


SMART VITICULTURE *by Dr. Richard Smart*

Thanks to Mark Kliewer for invaluable research

International viticultural researchers and grape and wine producers from the American wine sector attended a grapevine canopy management symposium at the University of California, Davis, on July 16, 2009. This symposium was dedicated to Professor Emeritus Mark Kliewer of UC Davis, who was a staff member there for more than 30 years and a pioneer in grapevine canopy management research in California.

Professor Kliewer organized the first international symposium on grapevine canopy management, and held in California in August 1986, attended by more than 500 people. Many of the speakers at that first symposium contributed to the second symposium 23 years later.

During his career, Kliewer made significant contributions to the California wine industry through his research on canopy management. Many of the present-day grape production systems can be traced to his research. He trained more than 50 graduate students, who are a continuing testament to his enthusiasm for viticultural research.

This symposium was organized by Dr. Nick Dokoozlian, one of Kliewer's former graduate students and currently vice president of Viticulture for E&J Gallo Winery, Modesto, Calif., and Jim Wolpert of the Department of Viticulture & Enology, at UC Davis.

"It is difficult for me to imagine a viticulturist that has had a more profound impact on modern California grape production than Dr. Kliewer, professor emeritus at UC Davis," says Dokoozlian. "Kliewer trained a generation of viticulturists that currently influences grape and wine production in every major growing region in the world.

"A prolific researcher, he also laid the foundation of knowledge necessary to establish many of the standard vineyard development and cultural practices used today, including nitrogen and potassium fertilization, irrigation, vine spacing, training/trellising systems and canopy management. It is nearly impossible to visit a vineyard today, in California or many other parts of the world, which has not been directly influenced by Kliewer's work.

This text reprinted from first publication: Nov./Dec. 2009.

"As a young graduate student, I found working alongside Kliewer in the vineyard quite challenging. He always volunteered for the most physically demanding job during harvest, and worked both quickly and methodically. His office door was always open to students and others requesting his assistance and advice. Hard working, dedicated, kind and humble, Kliewer's scientific accomplishments were overshadowed only by his outstanding personal character. It was a great privilege to study and work under his guidance, and I am very proud to call Mark Kliewer my mentor and friend."

Dr. Pat Bowen (Canadian viticultural scientist and a former Kliewer student), began the symposium, reviewing Kliewer's research contribution. His early work was in temperature-controlled growth cabinets where he showed the significance of light and temperature in the formation of organic and amino acids, sugars and anthocyanins.

By the 1980s, research conducted by Dr. Nelson Shaulis (Cornell University, Geneva, NY), in the eastern U.S. and European and Australian researchers demonstrated that the light and temperature environment of grape berries and leaves have an important impact on fruit composition and wine quality. This began a new period of research activity for Kliewer and his students, investigating aspects of vine physiology and canopy management in California.

Since the first symposium, the science has improved considerably. Dr. Hans Schultz, (director of the Geisenheim Research Institute, Geisenheim, Germany and also a graduate of UC Davis), presented results obtained from computer modeling of grapevine canopies. Using this approach, grapevine physiology can be simulated with a computer, allowing for evaluation of a wide range of inputs like climate and canopy architecture.

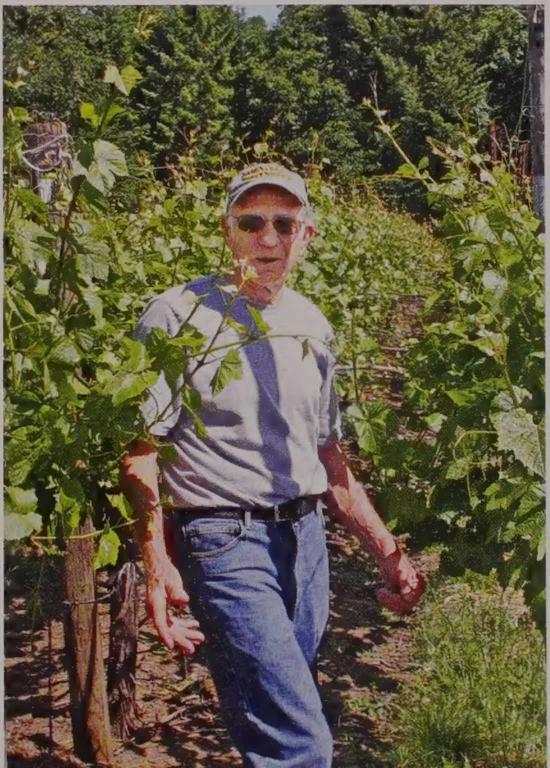
An Australian perspective on canopy management was presented by Dr. Peter Clingeleffer (CSIRO Plant Industry, Merbein, Victoria, Australia) who emphasized the important contributions of vineyard mechanization and minimal pruning.

Dr. Alan Lakso (professor and researcher at Cornell University's Geneva Experiment Station) was also a Kliewer graduate student. He presented intriguing results, showing the impact of the link between shoot growth rates and canopy microclimate on the concentration of methoxypyrazines in Cabernet Franc grapes in upstate New York.

Excessive green characters (sometimes called "veggie") are common in cool, wet climates like New York. It has long been known that fruit shading contributes to such undesirable character, but research by Lakso and his colleagues has shown that actively growing shoots in late July also contribute to these negative characters in grapes and wine.

Dr. Alain Carbonneau (professor of viticulture at Montpellier [France] SupAgro, and current president of GiESCO [Group of International Experts of Vitivinicultural Systems for CoOperation]) presented a description of grapevine trellis systems in use around the world and showed how the wide range of grapevine canopy types can be analyzed and modeled. In particular, a few simple measurements can tell much about how vineyards will perform.

Dokoozlian reviewed the past 20 years of canopy management practices in California, including trellising and variation in tractor row and vine spacing. Contrary to popular opinion, he reported that research has shown that decreasing spacing in the vine row creates greater canopy density, an effect greater than inter-vine competition. Other studies show that narrow tractor rows, a practice often proposed for the purpose of improving wine quality, also lead to increased yield.



Professor Mark Kliewer

Dokoozlian emphasized how vigor manipulation by irrigation management has become an important component of canopy management. He noted the biggest change in canopy management in California in the past 20 years has been the adoption of the vertical shoot positioned (VSP) system. VSP is best suited for low- to moderate-vigor situations, but unfortunately, it is also used in high-vigor situations.

Leaf removal in the fruit zone has now become widespread, and that improving fruit exposure with this practice helps reduce botrytis bunch rot. Regrettably, it is often done excessively, which is a particular problem in warm to hot growing regions where excessive exposure is enhanced by leaf removal in north-south oriented vine rows.

Italian researchers Dr. Stefano Poni (Istituto di Frutti-Viticoltura, Universita Cattolica del Sacre Cuore, Piacenza) and Dr. Cesare Intrieri (Department di Colture Arboree, University of Bologna) presented another aspect of leaf removal. They have shown that removal of mature leaves before bloom reduces fruit set and produces loose clusters (less prone to bunch rot). Because of regrowth of leaves by the vine and reduced yield, the improved ratio of leaf-area to fruit-weight leads to improved wine quality. The Italians have also taken advantage of Advantis vineyard mechanization, which requires less than 20 hours labor input per acre.

It was my pleasure to present the final paper, a particular honor since I was one of the speakers at the 1986 symposium. I went back some 130 million years to when flowering plants appeared in the Cretaceous period. One of 300 plant families that have subsequently been classified is Vitaceae, which contains *Vitis vinifera*, the European grapevine.

Many scientific members of the audience were surprised to learn that Charles Darwin, the founder of evolutionary theory, was a viticultural scientist. Darwin studied grapevines and other climbing plants in his glasshouse, which survives to this day at Down House near London.

Darwin was intrigued by the grapevine, particularly by the fact that opposite the leaf may exist a tendril or an inflorescence (which subsequently will become a grape cluster) or some intermediate form. Darwin wrote in 1865 "that these cases illustrate in a striking manner the principle of the gradual evolution of species."

Canopy management researchers are well aware that the grapevine is in a "fruiting mode" in a well-illuminated canopy, which is also important for wine quality. I made the point that, unfortunately, canopy management research is now not very fashionable, despite the substantial gains in yield and wine quality that are possible. Viticultural research topics like molecular biology and precision viticulture are much more popular today than canopy management.

I noted the commercial success of companies using my canopy management advice, in particular Pago Vallegarcia in Spain and Fortress Vineyards in Lake County, Calif. Both companies have improved profitability from increased yields and/or wine quality.

I regret that canopy management techniques are not more widely applied in commercial vineyards, especially in my home country of Australia. Because of research efforts by Kliewer and others, the adoption rate and awareness in the U.S., and particularly California, is much higher.

The symposium presented important research about the state of canopy management today, but will there ever be another canopy management seminar? I doubt it. Like many other fashions in the wine business, canopy management may have passed its "golden years." PWV

Dr. Richard Smart is rewriting and thoroughly revising Sunlight into Wine. *He is happy to receive statements from growers and winemakers about their commercial success with various aspects of canopy management. Interested persons should contact Dr. Smart by email at richard@smartvit.com.au. Dr. Smart visits the U.S. frequently, and consulting appointments can be made by email. See also www.smartvit.com.au.*

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